Contemporary Clinical Neuroscience

Bing-wen Soong
Mario Manto
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Trials for Cerebellar Ataxias

From Cellular Models to Human Therapies

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Contemporary Clinical Neuroscience

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ISSN 2627-535X ISSN 2627-5341 (electronic)
Contemporary Clinical Neuroscience
ISBN 978-3-031-24344-8 ISBN 978-3-031-24345-5 (eBook)
https://doi.org/10.1007/978-3-031-24345-5

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Despite the importance of the cerebellum in numerous brain functions, the scientific community still lacks effective treatments for most cerebellar ataxias, a group of disabling disorders affecting children, young adults, and the elderly. Encompassing practicing neurologists and physician scientists, the editors of the book share a common dream: getting the most treasurable holy grail by finding cures for our patients afflicted with ataxias.

Many trials have intended to lead the way to end cerebellar ataxias. This book provides a link between molecular mechanisms, pathogenesis, and therapies of cerebellar ataxias. The book provides a comprehensive assessment of the pre-clinical and clinical trials dedicated to cerebellar ataxias in the last 25 years. For the past decades, many scientists have poured much blood, sweat, and tears working toward these ends. With their collective efforts, we believe that the light at the end of the tunnel is in sight.

This is the first book fully dedicated to trials and therapies of cerebellar ataxias. The book comes at a time of major applications of genetic tools, neuroimaging, and other biomarkers, as well as innovative treatments.

We are particularly grateful to the experts who contributed to the book by providing detailed and up-to-date chapters on these advances, to the referees, and to our patients and families contributing to clinical research.

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Charleroi, Belgium — Mario Manto
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Contents

Part I
Basic Science of Cerebellum and Ataxias

Functional Anatomy of the Cerebellum

Izumi Sugihara, Yuanjun Luo, and Richard Nana Abankwah Owusu-Mensah

Abstract This chapter revises the neuronal connections and their functional consequences in the mammalian cerebellum based on a new understating of its basic comparative and circuit-level anatomy. The transverse lobular structure and the longitudinally striped arrangement are both essential in understanding the functional organization of the cerebellum. Cerebellar neuronal circuitry has been revealed at the level of single axons. The intricate distribution pattern of zebrin-positive and zebrin-negative Purkinje cells represents the longitudinally striped organization, which is linked to the topographic axonal projection patterns of climbing fibers and Purkinje cells. On the contrary, mossy fibers show distinct axonal projection patterns more or less related to lobules. The cerebellar outputs from different parts of the cerebellar nuclei project to the cerebellar-recipient thalamic nuclei and other targets to be involved in motor and non-motor functions. Tentatively, the cerebellar cortex has some nine divisions of different functional localization related to the region-specific axonal projection patterns.

Keywords Mouse · Marmoset · Human · Zebrin · Aldolase C · Climbing fibers · Mossy fibers · Purkinje cells · Functional localization

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_1

1 Introduction

This chapter aims to describe the standard functional anatomy of the cerebellum in a systematic concise way and to provide enough anatomical basis to understand the rest of the book. Many new findings, including output projections based on viral vector tracings, the longitudinally striped organization of the cerebellar cortex, and functional localization based on human imaging studies, have been covered within the context of the systematic description. However, morphological development is beyond the scope of this chapter.

For simplicity, we use "primate(s)" to mean "non-human primate(s)" in this chapter.

2 Macroscopic Anatomy of the Cerebellum

2.1 Outer Shape and Orientation

The human cerebellum is a dumbbell-shaped structure located inferior to the occipital lobe of the cerebrum and posterior to the brain stem. The human cerebellum is connected to the upright brain stem with horizontally running cerebellar peduncles in the anterior aspect. In the rodent, the cerebellum is roughly diamond-shaped and widely exposed in the dorsocaudal part of the brain. Its ventral aspect is connected to the horizontal brain stem with vertically-running cerebellar peduncles. Thus, the spatial orientation of the cerebellum is different, approximately 90° between the human and the rodent. Consequently, the anterior, posterior, superior, and inferior directions in the human cerebellar anatomy are the equivalence of ventral, dorsal, rostral, and caudal directions in the rodent cerebellar anatomy, respectively. However, the primate's cerebellum orientation is the intermediate between humans and rodents. The cerebellar orientation in mammals may therefore suggest an evolutional link in locomotion posture among mammals, from quadrupedalism to bipedalism in tetrapods. In the following, the terms "anterior, posterior, superior, and inferior" are used for the human cerebellum, whereas the terms "ventral, dorsal, rostral, and caudal" are used for the animal cerebellums (mainly for the rodent cerebellum).

The highly foliated cerebellar cortex unfolds to half the area of the cerebral cortex. However, it has only one-tenth the size of the cerebrum. In the posterior (dorsal, in animals) and inferior (caudal) aspects, the cerebellar surface is divided into the medial part (vermis) and the lateral parts (hemisphere, or paravermis and hemisphere) by the longitudinal dent in which the paravermal vein runs. These two divisions can be extended to the superior (rostral) aspects where the paravermal vein is absent. The whole lateral part may be designated as the hemisphere, as done usually in human cerebellum literature. Alternatively, suppose the topographic corticonuclear projection is considered. In that case, the lateral part is divided into the

paravermal area (or pars intermedia), which projects to the interpositus nucleus, and the hemisphere, which projects to the lateral (dentate) nucleus. However, there is no visible landmark (imaginary longitudinal line) in the cerebellar surface for the boundary between the paravermal and hemispheric parts. The characteristics described above are analogous in all mammals except for the relative sizes of the vermis and the hemisphere. The remarkably large hemisphere is reflected in the dumbbell shape in the human cerebellum.

2.2 The White Matter of the Cerebellum

The cerebellar white matter is the structure that connects the cerebellum to the brain stem and physically supports the entire cerebellum's integrity. The lobular organization of the cerebellar cortex is reflected in the arborization pattern of the white matter.

The major cerebellar white matter mainly runs transversely in the deepest portion of the cerebellum, mainly rostrally and partly dorsally to the cerebellar nuclei. This white matter is continuous to the middle cerebellar peduncles in the most lateral portion rostrolateral to the lateral nucleus. The middle cerebellar peduncle (or brachium pontis) is the pathway mainly for mossy fiber axons from the pontine nucleus and nucleus reticularis tegmenti pontis. Next, at the portion rostral to the lateral nucleus, this white matter is continuous to the inferior cerebellar peduncle. The inferior cerebellar peduncle (or the restiform body) is the pathway for almost all mossy fiber axons from the medulla and spinal cord and for climbing fiber axons (except for pontine-pathway spinocerebellar axons and some climbing fiber axons; see below). Ventral to this transversal bundle of the white matter, the longitudinally running bundle of the white matter starts from the hilus of the cerebellar nuclei, and its exit forms the superior cerebellar peduncle (or brachium conjunctivum). At the position where the superior cerebellar peduncle exits the cerebellum, white matter of a transverse axonal bundle covers the superior cerebellar peduncle dorsally. This transversal bundle contains a population of inferior olive (IO) axons and a pontine-pathway population of spinocerebellar axons, which preferentially project to the vermal area of the anterior lobe (Sugihara et al. 1999; Luo et al. 2018; Zhang et al. 2021). This axonal bundle is distinct from the superior cerebellar peduncle; it may be regarded as the rostral extension of the inferior cerebellar peduncle.

There is the maintenance of somatotopic lateralization in the spinal cord, dorsal column nuclei, and the cerebellum. However, the lateralization is opposite in the brain stem nuclei. With this, input axons originating from brain stem nuclei, except for the dorsal column nuclei, often cross the midline before entering the peduncle. Likewise, output axons also generally cross the midline after exiting the superior cerebellar peduncle.

2.3 Cerebellar Nuclei

The cerebellar nuclei are relatively small gray matter embedded in the deep central area of the cerebellar white matter. In the human and primate cerebellum, four nuclei—the fastigial nucleus (medial nucleus [MN]), globose nucleus (posterior interpositus nucleus), emboliform nucleus (anterior interpositus nucleus), and dentate nucleus (lateral nucleus)—are well separated from one another by the white matter. These nuclei are partly continuous with recognizable boundaries in rodents.

In humans, the dentate (lateral) nucleus is much larger than other nuclei and consists of a bag-shaped single sheet with numerous indentations. The anterior (microgyric) part of the dentate nucleus has a finer indentation than the posterior (macrogyric) part (Yamaguchi and Goto 1997).

2.4 Cerebellar Lobules in the Vermis

The degree of cerebellar surface foliation is significantly different among mammals. However, the major (deep) foliation pattern of the vermis has a high similarity among all mammals in the midsagittal section (Fig. 1a). Comparative morphological studies by Larsell (1970), and Larsell and Jansen (1972), have defined ten lobules (lobules I–X, from the rostral end to the caudal end) in the vermis of various mammals and humans. Subsequent publications adopted Larsell's definition (human: Schmahmann et al. 1999; macaque: Madigan and Carpenter 1971; Paxinos et al. 2009; marmoset: Fujita et al. 2010; Paxinos et al. 2011; rat: Voogd 2004; Paxinos and Watson 2017; Swanson 1998; mouse: Marani and Voogd 1979; Paxinos and Franklin 2019; Fujita et al. 2014).

Comparative identification of vermal lobules is rather straightforward based on their relative size, shape, and position across various mammals, including rodents, primates, and humans (Luo et al. 2017). They are divided into two groups, lobules I–V (anterior lobe) and lobules VI–X (posterior lobe), by the most profound fissure described as the primary fissure. Each of the two groups is then divided into individual lobules by other deep fissures, except that fissures between lobules I and II, between lobules IV and V, and between vermal lobules VI and VII are not as deep as others. Since the fissures that divide these neighboring lobules are shallow, these neighboring lobules are sometimes regarded as a combined single lobule (lobules I and II, lobules IV and V, lobules VI and VII). Indeed, "lingula cerebelli" is regarded as composed of lobules I and II, which are not distinguishable in the human cerebellum.

Vermal lobules VI and VII are divided into multiple sublobules (folia) by relatively shallow fissures. The subdivisions of lobules VI and VII are complicated due to the inconspicuous fissure between them, and inconsistent foliation patterns in these lobules among mammals. Therefore, there may be some inconsistency in the definition and the nomenclature of lobules among different mammalian species. We have proposed that rodent (sub)lobule VIa and (sub)lobules VIb–c + VII are

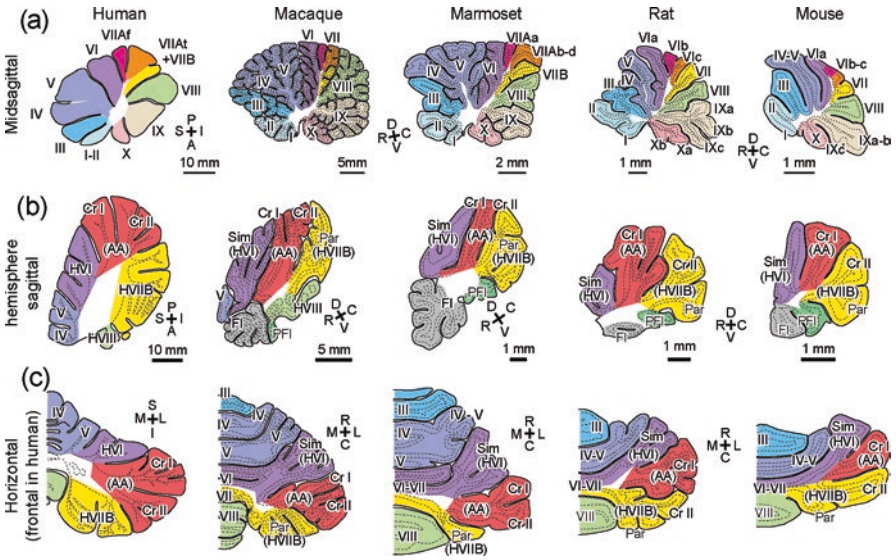

Fig. 1 Lobular organization of mammalian cerebellums. (**a**–**c**) Midsagittal (**a**), hemispheric sagittal (**b**), and horizontal (**c**) sections of the human, macaque (*Macaca mulatta*), marmoset (*Callithrix jacchus*), rat (Long-Evans), and mouse (C57BL/6N) cerebellums (from left to right). (Putatively) homologous lobules are labeled in the same color in the central vermal area. Note inconsistent names in lobules VI–VII between primates and rodents. Human and mouse section drawings in (**a**) were based on our samples. Abbreviations: I–X, lobules I–X; A, anterior; AA, ansiform area; C, caudal; Cr I, crus I; Cr II, crus II; D, dorsal; Fl, flocculus; HVI–HVIII, hemisphere lobules VI–VIII; I, inferior; L, lateral; M, medial; P, posterior; Par, paramedian lobule; PFl, paraflocculus; R, rostral; S, superior; Sim, simple lobule; V, ventral. (Marmoset and rat drawings in (**a**) were based on the data used in the figures of Fujita et al. (2010). The macaque section in (**a**) was redrawn from Larsell (1953). Drawings in (**b**) and (**c**) were modified from Luo et al. (2017). The lobule nomenclature is derived from Schmahmann et al. (1999) for human, Paxinos et al. (2009) for macaque, Paxinos et al. (2011) for marmoset, Paxinos and Watson (2017) for rat, and Paxinos and Franklin (2019) for mouse)

equivalent to primate (and human) lobules VI and VII in the vermis, respectively (Fujita et al. 2010; purple, red, orange, and yellow areas in Fig. 1a).

For correct identification of lobules among different animal species, not only position and shape but also afferent and efferent connection patterns and longitudinally striped patterns should be considered.

2.5 Lobules in the Hemisphere

As in the vermal lobules, the basic organization in the hemispheric lobules is regarded as commonly shared by all mammals (Bolk 1906; Larsell 1970). However, species-dependent differences in lobular structure are greater in the hemisphere compared to the vermis.

The hemispheric lobules are lateral protrusions of the vermal lobules in the case of lobules I–VI, except that lobule I hardly forms a lateral protrusion and that lobule II protrudes to a limited extent. Since lobule VI (or lobule VIa in rat and mouse) simply extends laterally as compared with the caudally neighboring crus I (below), it has been designated as simple lobule (Bolk 1906). The same hemispheric lobule is also designated as "hemispheric lobule VI (HVI)" (Larsell 1970; Schmahmann et al. 1999) in primates and humans.

Crus I and crus II (terms originating from Bolk 1906) are the two major lobules expanded most laterally in the central part of the cerebellar hemisphere in all mammals including humans. The paramedian lobule or lobule HVIIB is located caudal to crus II. Then, the apparently most caudal hemispheric lobule is the lateral extension of vermal lobule VIII, named "copula pyramidis" in rodents, "sublobule p of the paramedian lobule" in primates, and lobule HVIII in humans. The outer shape of these hemispheric lobules (crus I, crus II, HVIIB, HVIII) is significantly different among mammals and humans. Concerning the definition of hemispheric lobules, we have proposed that crus I in the mouse and rat is homologous with the combination of crus I and crus II in primates and humans (designated as the "ansiform area"; Luo et al. 2018). In the human cerebellum, crus I, crus II, lobule VIIB, lobule VIIIA, and lobule VIIIB have been defined in the posterior hemisphere based on thorough three-dimensional observation of lobule and fissure structures in magnetic resonance imaging (MRI) data and comparison with earlier nomenclatures (Schmahmann et al. 1999). However, the homology between human and primate cerebellar lobules in this area does not seem to be fully obvious. The ansoparamedian fissure that separates lobule HVIIB from crus II is less clear than the prepyramidal/prebiventer fissure that separates lobule HVIIB from lobule HVIIIA in the human cerebellum. Therefore, there seems to be the possibility that the combination of crus II and lobule HVIIB in the human cerebellum may be homologous to primate crus II. Axonal connection analysis by tractography from an MRI image (Steele et al. 2017) would provide some information about lobule homology in the future. The volume of the ansiform area relative to the whole cerebellum increases significantly in dexterous mammals (Luo et al. 2018; Fig. 1b,c). In the human cerebellum, the increased volume of the ansiform area makes the remarkable expansion of the cerebellar hemisphere.

The paraflocculus (lobule HIX) and flocculus (lobule HX) are generally regarded as the hemispheric part of lobules IX and X, respectively. However, their cortices are joined and connected to the caudolateral edge of HVIII rather than vermal lobules IX and X in the mammalian cerebellum. In the human cerebellum, the paraflocculus occupies most of the tonsil, which is located at the medial corner between the medulla and posterior vermis. In rodents and other animals, the paraflocculus is a peculiar bulb-like protrusion in the ventrolateral part of the cerebellum (Panezai et al. 2020). This protrusion fits into the arcuate fossa surrounded by the anterior semicircular canal (Panezai et al. 2020). The flocculus is located ventromedial to the paraflocculus at the lateral or rostrolateral junction between the medulla and the cerebellum. In humans and primates, the flocculus is separately protruded from the paraflocculus, whereas they are neighboring in rodents.

2.6 *Unfolded Schemes of the Cerebellar Cortex*

The deeply foliated surface of the cerebellum forms a continuous single sheet of the cerebellar cortex. Consequently, the entire cerebellar cortex can be shown schematically in an unfolded scheme in the two-dimensional space. Early unfolded schemes were used to show the positional relationship of lobules and map the somatotopic representation (Bolk 1906; Larsell 1970; Snider and Eldred 1952). Later, two-dimensional unfolded schemes that reflected actual lobular size and continuity were created for various mammals (mouse: Fujita et al. 2014; Sarpong et al. 2018, Fig. 2c; rat: Sugihara and Shinoda 2004; Ruigrok 2003; marmoset: Fujita et al. 2010, Fig. 2b; human: Diedrichsen and Zotow 2015, Fig. 2a). Since the original three-dimensional shape of cerebellar lobules is complicated, some deformation is inevitable in forming any two-dimensional scheme. Nevertheless, unfolded schemes are useful in mapping and comparing lobular dimensions. The lobular stripe patterns (Fig. 2b,c), distribution of axonal terminals, longitudinal stripe patterns, local activities recorded in functional MRI (fMRI), and schematic of functional localization can be mapped in these two-dimensional schemes, facilitating understandability.

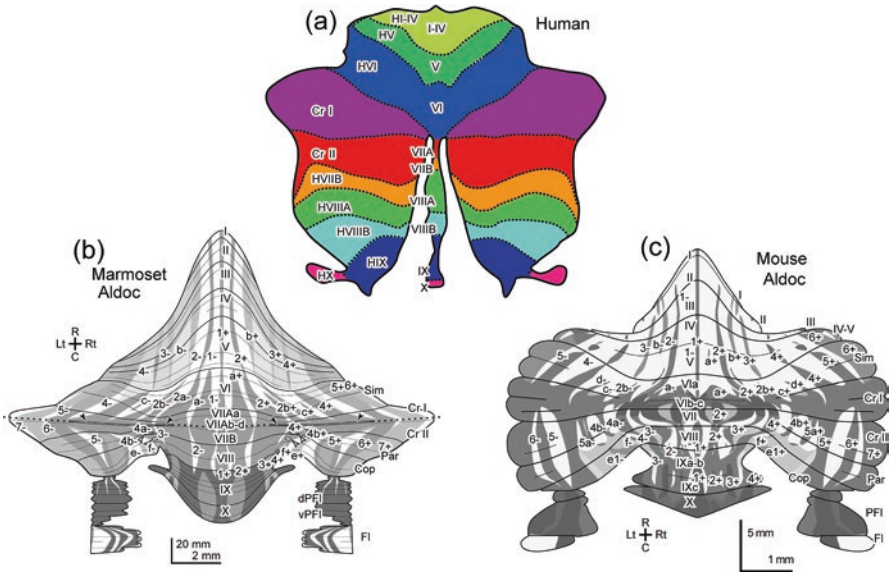

Fig. 2 Unfolded schemes of the cerebellar cortex of human (**a**), marmoset (*Callithrix jacchus*) (**b**), and mouse (**c**). Abbreviations: I–X, lobules I–X; a–c, A, B, sublobules a–c, A, B; C, caudal; Cop, copular pyramids; Cr I, crus I; Cr II, crus II; dPFl, dorsal paraflocculus; Fl, flocculus; HI–HX, hemispheric lobules I–X; Lt, left; PFL, paraflocculus; Par, paramedian lobule; R, rostral; Rt, right; Sim, simple lobule; vPFl, ventral paraflocculus. (The human scheme is based on Diedrichsen and Zotow (2015) with permission. The marmoset and mouse schemes, in which the zebrin pattern is mapped, are reproduced from Fujita et al. (2010) and Sarpong et al. (2018))

3 Neuronal Components and Circuitry of the Cerebellum

The cerebellar cortex is composed of three layers—molecular layer, Purkinje cell (PC) layer, and granular layer, from the surface to the deep white matter. Neuronal components are uniquely positioned in these layers. The basic neuronal components and neuronal circuit organization, which have been long known in the cerebellar cortex (Ramón y Cajal 1911; Eccles et al. 1967; Fig. 3a), are uniform throughout the

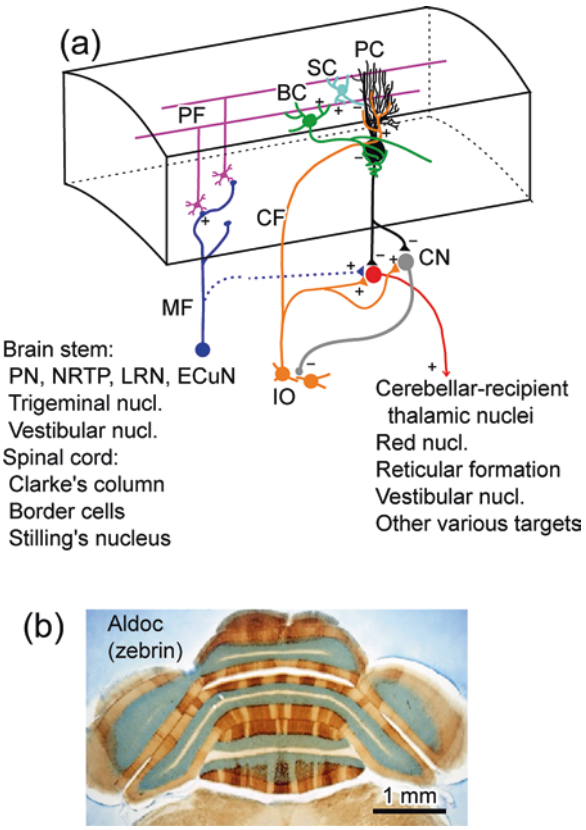

Fig. 3 Neuronal composition of the cerebellar cortex. (**a**) Schematic drawing of neuronal components and their basic synaptic connections in the cerebellar cortex. "+" and "−" indicate excitatory and inhibitory synaptic connections. (**b**) Aldolase C (Aldoc, zebrin) expression in Purkinje cells. An image of Aldoc immunostaining in a coronal section of the mouse caudal cerebellum is shown. Brown, immunostaining reaction; Blue, thionine counterstaining. Abbreviations: BC, basket cell; CF, climbing fiber; CN, cerebellar nucleus; ECuN, external cuneate nucleus; IO, inferior olive; LRN, lateral reticular nucleus; MF, mossy fiber; NRTP, nucleus reticularis tegmenti pontis; PC, Purkinje cell; PN, pontine nucleus; PF, parallel fiber; SC, stellate cell

cerebellar cortex. However, some regional variations exist in some detailed aspects. Different cell types and their regional variations have been confirmed in a transcriptomic analysis (Kozareva et al. 2021).

3.1 Purkinje Cells and Climbing Fibers

Purkinje cells are the sole output component of the cerebellar cortex. Their somata are placed in a two-dimensional sheet, i.e., the Purkinje cell layer, in close proximity to each other. They have round soma, generating an axon from the basilar pole and one or two thick dendrite(s) from the apical pole. The Purkinje cell dendrite forms a fan-shaped dendritic arbor by multiple branching (Fig. 4a). By mechanisms that are not entirely clarified, the Purkinje cell dendritic arbor is usually strictly arranged in a single sheet in which the domain of each dendritic branch does not overlap with each other. The dendritic field is classified into two areas of different innervation: proximal dendrites, which indicate the thick proximal parts of the dendritic arbor, receive the climbing fiber innervation, whereas distal dendrites, which indicate the thin distal parts of the dendritic arbor, receive the parallel fiber innervation at dendritic spines.

One or a few local recurrent collaterals that distribute in the Purkinje cell layer and superficial granular layer (Fig. 4a) have been observed in 92% of Purkinje cells (Sugihara et al. 2009). The target area of most Purkinje cell axons is a localized part of the ipsilateral cerebellar nucleus (CN) (Fig. 4b–d). Some Purkinje cell axons or axonal branches project to extracerebellar targets such as vestibular nuclei and the parabrachial nucleus.

A Purkinje cell is innervated by one climbing fiber, which is one of the major input axons in the cerebellar cortex originating from the inferior olive (Llinás 2014). A climbing fiber reaches the soma of a Purkinje cell from the granular layer, giving rise to sparse terminals around the soma. Great branching and dense termination of a climbing fiber start at the rise of proximal dendrites (Fig. 4h, j). One climbing fiber has as many as 250 terminals (rat: Sugihara et al. 1999) and produces a large synaptic current in Purkinje cell proximal dendrites. Thus, an action potential of a climbing fiber triggers a complex spike response in the innervated Purkinje cell, in contrast to simple spikes, which are intrinsic action potentials generated at the axon initial segment of the Purkinje cell.

Given the molecular expression profile and basic physiological properties, Purkinje cells are grouped into heterogeneous populations (Viet et al. 2021). The most frequently mentioned populations are zebrin (aldolase C)-positive and zebrin-negative populations that are distributed in alternating longitudinal stripes in the cerebellar cortex (Fig. 3b).

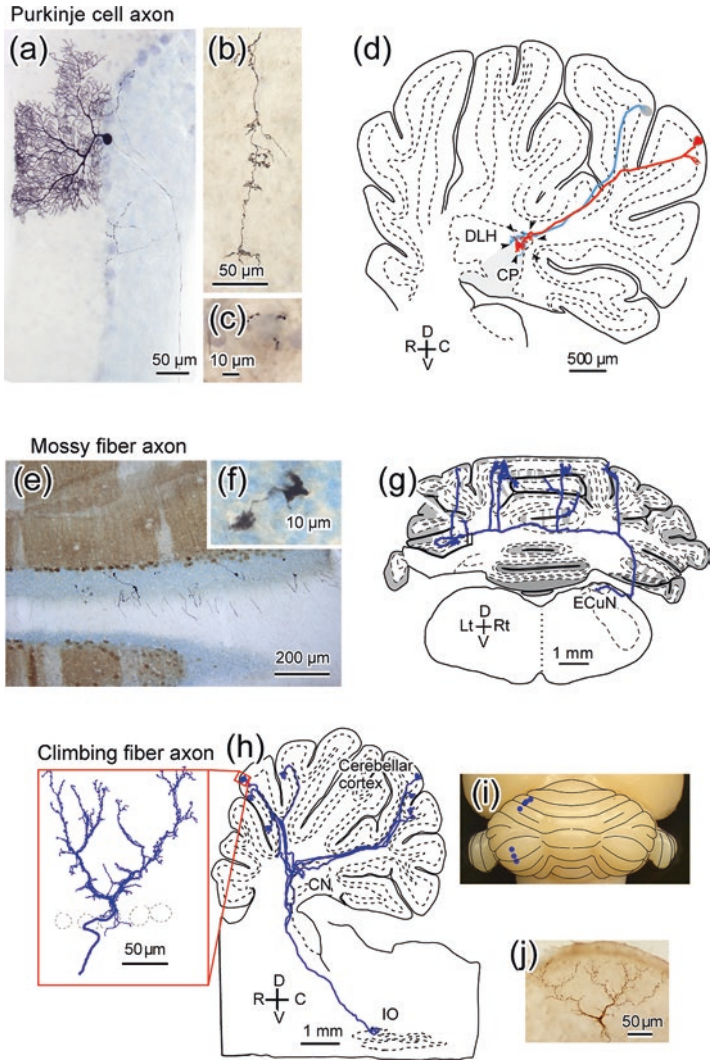

Fig. 4 Afferent and efferent projections of the cerebellum. (**a**–**c**) Images of Purkinje cell axons of the rat. Dendritic arbor, soma, and axon collaterals (**a**), terminal arbor in the cerebellar nucleus (**b**), and terminals that surround a cerebellar nucleus neuron (**c**) are shown (Images and data are reproduced from the original data of the study published in Sugihara et al. (2009)). (**d**) Lateral view of two reconstructed single Purkinje cell axons that terminate in the dorsolateral hump of the anterior interpositus nucleus. (**e**) Image of labeled mossy fiber axons originating from the external cuneate nucleus of rat. (**f**) Magnified image of mossy fiber terminals of spinocerebellar axons of mouse. (**g**) Reconstructed single mossy fiber axon originating from the external cuneate nucleus of rat (Images and data in (**f**) and (**g**) are reproduced from the original data of the study published in Quy et al. (2011)). (**h**) Reconstructed single climbing fiber axon of rat. (**i**) Distribution of the axon shown in (**h**) in the whole-mount image of the rat cerebellum. (**j**) Image of labeled climbing fiber terminal (Images and data in (**h**)–(**j**) are reproduced from the original data of the study published in Sugihara et al. (1999)). Abbreviations: C, caudal; CN, cerebellar nucleus; CP, caudal pole of the lateral nucleus; D, dorsal; DLH, dorsolateral hump of the anterior interpositus nucleus; ECuN, external cuneate nucleus; IO, inferior olive; Lt, left; R, rostral; Rt, right; V, ventral

3.2 Molecular Layer Interneurons

Basket cells (BCs) and stellate cells (SCs) are GABAergic neurons designated together as "molecular layer interneurons," but generally morphologically distinguishable from each other (Sultan and Bower 1998). Somata of basket cells and stellate cells are located in the deeper one-third and outer two-thirds of the molecular layer, respectively (Brown et al. 2019). Their dendrites receive an excitatory synaptic connection from parallel fibers. They also receive "spill-over" excitation by climbing fibers (Malhotra et al. 2021). Their axons form local arborization and innervate multiple Purkinje cells at the soma (in case of basket cells) and the dendrite (in case of stellate cells). The terminal arbor of basket cells densely surrounds single Purkinje cells at the soma and particularly at the initial axonal segment (called "pinceau": Ramón y Cajal 1911), enabling effective inhibition of Purkinje cell activity through GABA release and electrical field interaction (ephaptic inhibition: Blot and Barbour 2014). Stellate cell activity decreases the firing regularity of Purkinje cell simple spikes, whereas basket cell activity decreases the firing frequency of Purkinje cell simple spikes (Brown et al. 2019).

3.3 Granule Cells, Parallel Fibers, and Mossy Fibers

The granular layer is densely packed with granule cells. They have small soma, three to five short dendrites, and a vertical axon. Mossy fibers are the most abundant input in the cerebellar cortex, originating from diverse areas and nuclei. A mossy fiber has large ellipse-shaped terminals (long diameter, ~10 μm) with many irregular bulges and dents ("rosette" terminal) at its ends, branching points, and path (Fig. 4e, f). A rosette terminal is densely packed by dendrites of multiple nearby granule cells, to which it makes a synaptic contact, forming the structure called "glomerulus."

Axons of granule cells are non-myelinated and run straight upward to the molecular layer in which it bifurcates into a parallel fiber in a T-shape. The parallel fiber runs straight in the transverse direction in the molecular layer for a length of about 2 mm in the mouse (Huang et al. 2006), which can cross several zebrin stripes (see later section). The ascending granule cell axon and the parallel fiber make en-passant synaptic contacts to Purkinje cell distal dendrites and dendrites of the stellate, basket, and Golgi cells. The molecular layer is densely packed by (1) a bundle of parallel fibers, (2) plates of Purkinje cell dendritic arbors, which cross parallel fibers at a right angle (Fig. 3a), (3) stellate and basket cells and Golgi cell dendrites, and (4) processes of Bergmann glia cells.

Parallel fibers do not make functional synaptic contact with all Purkinje cells they cross. Furthermore, the population of granule cells that forms effective synaptic contact with a given Purkinje cell through the parallel fiber–Purkinje cell synapse is distributed in patches in the nearby granular layer (Spaeth et al. 2022).

3.4 Golgi Cells and Other Inhibitory Cells in the Granular Layer

Golgi cells are the most abundant type of inhibitory interneurons in the granular layer, although their number is about one-thousandth of the number of granule cells (D'Angelo and Casali 2013). They are located at any depths of the granular layer but more frequently in the superficial than in the deep granular layer. They have basal and apical dendrites to receive excitatory inputs from parallel fibers and ascending granule cell axons (Cesana et al. 2013) and from mossy fiber terminals. Their inhibitory inputs are from mixed GABA-glycinergic nucleocortical projection neurons (Ankri et al. 2015) and possibly from other Golgi cells, Lugaro cells, and Purkinje cell axon collaterals. Golgi cells also receive dendrodendritic electrical synapses from thier kind nearby and project their axons to granule cell dendrites in the glomerulus.

Lugaro and globular cells are GABAergic neurons located in the superficial granular layer, often immediately underneath the Purkinje cell layer, whereas candelabrum cells are GABAergic neurons located in the Purkinje cell layer (Lainé and Axelrad 1994). Lugaro cells are fusiform-shaped and smaller than Purkinje and Golgi cells in size, while globular cells are round-shaped and smaller than Lugaro cells. These three types of cells receive Purkinje cell collaterals and other inputs and project to dendrites of molecular layer interneurons. In addition, Lugaro cells receive climbing fiber axon collaterals and project to Golgi cell dendrites in the molecular layer.

Unipolar brush cells are excitatory local neurons in the granular layer of the nodulus and flocculus. They have a single brush-shaped large and short dendrite contacting a mossy fiber and giving rise to a local mossy fiber axon.

3.5 Glial Cells in the Cerebellar Cortex

The molecular layer of the cerebellar cortex contains a specific organization of glial cells. Bergmann glial cells are specialized astrocytes located mainly in the Purkinje cell layer and occasionally in the deep molecular layer near the Purkinje cell layer. They have multiple vertical processes that ascend the molecular layer straight to reach the surface of the cerebellar cortex. Abundant shorter processes are given from the cell body and the vertical process. NG2-glia cells (or oligodendrocyte precursor cells) are another population of glial cells in the molecular layer (Lin et al. 2005). Glia cell populations in the granular layer, white matter, and cerebellar nuclei are not remarkably different from those in other gray matter or white matter of the brain.

3.6 Neurons in the Cerebellar Nuclei

The cerebellar nuclei contain various types of excitatory and inhibitory neurons. They are heterogeneous regarding morphology, projection, neurotransmitter, and molecular expression (Uusisaari et al. 2007; Fujita et al. 2020). The distribution of types of neurons in the cerebellar nuclei is partly related to the compartmentalization determined by the topography of the Purkinje cell axonal projections.

The major group consists of glutamatergic output neurons, classified into heterogeneous populations based on differences in size, location, molecular expression profiles, electrophysiological properties, topographic innervation, and projection patterns. These properties are correlated and define five populations in the mouse medial nucleus (Fujita et al. 2020).

Inhibitory neuronal populations consist of GABAergic neurons that project to the inferior olive, glycinergic neurons that project to the brain stem (Bagnall et al. 2009), and mixed GABA-glycinergic neurons that project to the cerebellar cortex, mainly to Golgi cells (Ankri et al. 2015).

4 Afferent Axonal Projections of the Cerebellum

Compared to the abundant axonal projections between areas in the cerebral cortex, the cerebellum does not have any long-distance intercortical projections of cortical neurons. Thus, all long-distance axonal projections in the cerebellum are described as the afferent (input) and the efferent (output). Their topographic patterns characterize the morphological organization of afferent and efferent projections. In other words, both the cerebellar cortex and nuclei are characterized by their compartmentalization related to the afferent and efferent axonal projection patterns. In the cerebellar cortex, compartmentalization is represented by transverse lobules and longitudinal stripes (Sugihara 2021).

4.1 Climbing Fibers Originating from the Inferior Olive

The inferior olive is the multilamellar nucleus located in the caudal and medial medulla immediately dorsal to the pyramidal tract. Virtually, all neurons of the inferior olive project to the cerebellum as climbing fibers. A single inferior olive axon branches into seven climbing fibers on average in the rat and gives rise to collaterals to terminate in the cerebellar nucleus (Sugihara et al. 1999). Climbing fibers originating from a single olivary axon usually terminate in a longitudinally band-shaped area in a single lobule, multiple neighboring, or separate lobules (Sugihara et al. 2001). It is often observed that some branches project to rostral lobules and other branches project to caudal lobules from a single olivary axon (lobules I–VIa versus

lobule VIII, or simple lobule versus crus II and paramedian lobule; Sugihara et al. 2001). When they terminate in multiple lobules, the longitudinal band-shaped termination areas in different lobules are located in a similar mediolateral position (Sugihara et al. 2001; Fig. 4h, i).

Thus, the climbing fiber signals are provided directly to specific Purkinje cells located in strictly defined areas and produce significant modulatory and plastic effects in the target Purkinje cells. Therefore, the topographical projection patterns of inferior olive afferents and olivocerebellar climbing fiber axons seem to have an important role in producing functional localization of the cerebellum.

4.2 Mossy Fiber Axons

Mossy fibers are the largest population of axons in the cerebellar white matter. Most mossy fiber axons originate from the so-called precerebellar nuclei in the brain stem and the spinal cord. The major precerebellar nuclei are the pontine nucleus and nucleus reticularis tegmenti pontis, both located in the pons, the lateral reticular nucleus, and the external cuneate nucleus, also located in the medulla. Almost all neurons in these nuclei project to the cerebellum as mossy fiber axons. Besides, the vestibular nuclear complex, the trigeminal nucleus, the gracile and cuneate nucleus, and medullary and pontine reticular formation contain many neurons that give rise to mossy fiber axons. In the spinal cord, the central cervical nucleus in the cervical segments, Clarke's column nucleus in the thoracic and upper lumbar segments, border cells in the ventral horn of lumbar segments, and Stilling's nucleus in the sacral segments are the main sources of mossy fibers (Luo et al. 2017, 2020; Zhang et al. 2021). However, many other populations of spinocerebellar neurons exist in various areas of the spinal cord gray matter, e.g., marginal Clarke's column neurons in the thoracic segments (Luo et al. 2017).

Mossy fiber axons usually innervate only the cerebellum; in other words, they do not branch before entering the cerebellum, although some lateral reticular nucleus and spinal cord axons have collaterals innervating the vestibular nucleus and other brain stem nuclei. In the cerebellum, the mossy fiber axons give rise to many collaterals that terminate in multiple lobules and multiple stripes, bilaterally or unilaterally. They possess mossy fiber termination as the main axonal arbor (precerebellar-type mossy fiber axons; Fig. 4g). The number of mossy fiber terminals per axon generally ranges between 50 and 150 in the precerebellar-type axons in the mouse and rat (Wu et al. 1999; Quy et al. 2011; Luo et al. 2018; Biswas et al. 2019; Na et al. 2019; Fig. 4g). The terminals originating from a single axon are often widely and sparsely spread but sometimes aggregated in a small cortical area (Luo et al. 2018). Terminals of mossy fibers arising from the same and different origins converge on the dendrite of a granule cell (Huang et al. 2013). The distribution of the mossy fiber terminals is well topographically associated with the transverse lobular longitudinal stripe organization of the cerebellar cortex. The topography of the mossy fiber projection pattern is quite specific to distinct mossy

fiber projections from different origins. Some of the mossy fiber axons project to the cerebellar nucleus with a topographic relationship, whereas some others do not project to the cerebellar nucleus.

There are distinct types of axons that possess a small number of collaterals that enter the cerebellar cortex and terminate as mossy fibers (Luo et al. 2018). Mossy fiber branches of vestibular primary afferent axons (Ando et al. 2020) and mossy fiber branches of the output axons of the cerebellar nucleus neurons belong to this type (non-precerebellar-type mossy fiber axons).

4.3 Distribution Pattern of Major Mossy Fiber Axons in the Cerebellar Cortex

Mossy fiber axons arising from various origins, which convey specific information, have different projection patterns (Fig. 5a). These projections are supposed to significantly contribute to the somatotopy (Fig. 5b) and functional localization of the cerebellar cortex (Sect. 7). Seminal tracing studies with Nauta's method performed mainly in cat in the 1960s and 1970s by Grant (1962), Brodal and Hoivik (1964), and others have clarified the pathways and terminal distribution patterns of mossy fibers originating from major sources in the brain stem and the spinal cord (cf. Brodal 1981; Schmahmann et al. 2019). Recently, analyses of single axon morphology have revealed branching patterns and collateral projections in mossy fiber axons originating from several sources (Wu et al. 1999; Quy et al. 2011; Luo et al. 2018; Biswas et al. 2019; Na et al. 2019; Luo et al. 2020; Ando et al. 2020; Zhang et al. 2021).

Mossy fiber projections from the spinal cord, dorsal column nuclei, and lateral reticular nucleus share relatively similar lobular projection patterns (Wu et al. 1999; Quy et al. 2011; Luo et al. 2018, 2020; Zhang et al. 2021), although they have different stripe preference (Gravel and Hawkes 1990). They project to vermal and paravermal areas of lobules I–V, rostral part of lobule VIa, and lobules VIII–IX, contributing to the somatotopy of limbs, trunk, and neck in these lobules.

Mossy fibers from the trigeminal nucleus mainly project to the simple lobule, crus I, crus II, paramedian lobule, and vermal lobule IX (Welker 1987; Van Ham and Yeo 1992), contributing to the somatotopy of the face in these lobules. Mossy fibers from the medial vestibular nucleus project preferentially to lobules IXc–X and flocculus, moderately to lobule I, and sparsely to all vermal lobules (Ando et al. 2020). Mossy fibers from other parts of the vestibular nuclear complex, including nucleus X, also project to the cerebellum, lobules I–V in particular (Matsushita and Okado 1981). Mossy fibers from the primary vestibular sensory afferent project to lobules I, IXc, and X (Brodal 1972).

The pontine nucleus sends its axons most broadly in the cerebellar cortex among precerebellar nuclei, covering all cerebellar lobules but lobule X (Brodal 1981; Biswas et al. 2019). The pontocerebellar projection shows lobule-related topographic organization (Brodal 1979; Brodal and Bjaalie 1992). The topography of

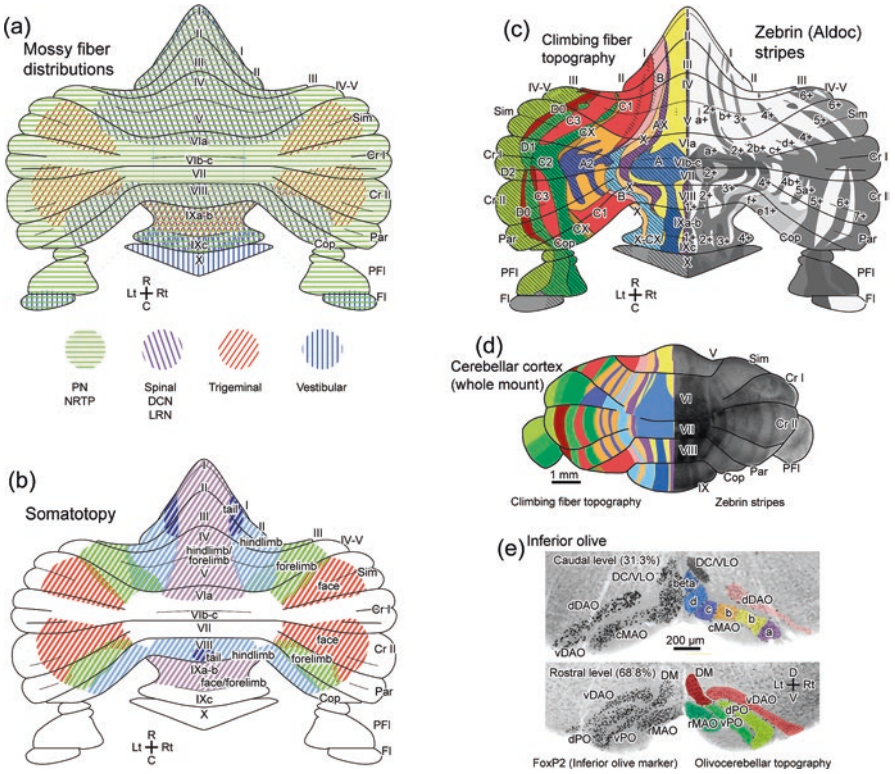

Fig. 5 Topographic distribution of mossy and climbing fibers in the cerebellar cortex. (**a**) Schematic distribution patterns of mossy fibers originating from the major origins—based on data published in rat and mouse single axon studies (Wu et al. 1999; Quy et al. 2011; Luo et al. 2017, 2020; Biswas et al. 2019; Na et al. 2019; Ando et al. 2020; Zhang et al. 2021) and tracing studies in cat and macaque in the 1960s and 1970s by Grant (1962), Brodal and Hoivik (1964), and others (cf. Brodal 1981; Schmahmann et al. 2019). (**b**) Schematic mapping of somatotopy in the cerebellar cortex (Cited from Khoa et al. (2021)). (**c**) Schematic distribution patterns of climbing fibers originating from subareas of the inferior olive—based on data published in rat and mouse studies (Sugihara and Shinoda 2004; Sugihara and Quy 2007). The right side shows the zebrin stripe pattern (Sarpong et al. 2018) for comparison. (**d**) Mapping of the climbing fiber distribution pattern drawn on the whole-mount preparation of the Aldoc-Venus mouse cerebellum (Modified from Luo and Sugihara (in press)). (**e**) Subareas of the inferior olive color-coded to show the topographic projection pattern of climbing fibers between (**c**) and (**e**), and (**d**) and (**e**); mapped on the FoxP2 immunostaining image of the mouse inferior olive (Modified from Luo and Sugihara (in press)). Abbreviations: 1+, 1−, and so on, compartments 1+, 1−, and so on; I–X, lobules I–X; a–d, sublobules or subareas a–d; A, B, C1, C2, C3, CX, D0, D1, D2, X, X–CX, names of cerebellar modules; beta, beta subnucleus of the inferior olive; C, caudal; cMAO, caudal part of the medial accessory olive; Cop, copular pyramids; Cr I, crus I; Cr II, crus II; dDAO, dorsal fold of the dorsal accessory olive; DC, dorsal cup; DM, dorsomedial subnucleus of the inferior olive; Fl, flocculus; LRN, lateral reticular nucleus; Lt, left; NRTP, nucleus reticularis tegmenti pontis; Par, paramedian lobules; PFl, paraflocculus; PN, pontine nucleus; PO, principal olive; R, rostral; Rt, right; Sim, simple lobule; vDAO, ventral fold of the dorsal accessory olive; VLO, ventrolateral outgrowth; vMAO, ventral part of the medial accessory olive

the pontocerebellar projection is simply summarized into three groups in the mouse (Biswas et al. 2019), although further analysis may distinguish more groups. Pontine nucleus axons originating from the rostral, medial, and lateral parts, which receive projections from association cortices of frontal, parietal, and temporal lobes (Schmahmann and Pandya 1997), terminate mainly in the paraflocculus, crus I (equivalent to crus I and crus II in primates: Luo et al. 2017), and lobules VIb–c and VII. Those originating from the central part of the pontine nucleus, which receives projections from the somatosensory and motor cortices of the face and forelimb somatotopy (Leergaard et al. 2000), terminate mainly in the simplex lobule, crus II, and paramedian lobule. Those originating from the caudal part of the pontine nucleus, which receives projections from the somatosensory and motor cortices of trunk and hindlimb somatotopy (Leergaard et al. 2000), terminate mainly in lobules II–VIa, VIII, and copula pyramidis. A single axon often innervates the above combination of lobules simultaneously by the interlobular axonal branching, indicating a functional link between these lobules.

4.4 Other Afferent Projections to the Cerebellar Cortex

Besides climbing and mossy fibers, the cerebellar cortex receives noradrenergic and serotoninergic projections from the brain stem. Noradrenergic axons originate from the locus coeruleus (Carlson et al. 2021), whereas serotoninergic axons originate from diverse brain stem regions and nuclei (Kerr and Bishop 1991). These axons are densely distributed in the cerebellar nuclei and all layers of the cerebellar cortex. In the molecular layer, serotoninergic fibers tend to run in the transversal direction, whereas noradrenergic fibers run in the longitudinal direction (Longley et al. 2021). These axons have "beaded" abundant varicosities along their path to release noradrenaline and serotonin. All neurons have receptors to these monoamines and produce responses when tested. At the behavior level, these neurotransmitters significantly modulate plasticity and learning in motor and cognitive behaviors. The innervation morphology of these axons also shows significant plastic changes (Nedelescu et al. 2017).

Some part of the cerebellum that receives orexigenic projection from the lateral hypothalamus has been reported. Some mossy fibers originating from the vestibular nucleus contain acetylcholine (Barmack et al. 1992).

4.5 Afferents of the Cerebellar Nuclei

Purkinje cell axons project to a small area of the cerebellar nuclei to form the main synaptic inputs for both excitatory and inhibitory output neurons in the cerebellar nuclei (Fig. 4c), although they may project to a broader area in the medial nucleus and the vestibular nuclei (Sugihara et al. 2009).

Almost all climbing fiber axons have collaterals terminating in the cerebellar nucleus (Fig. 4h). The termination area of collaterals of a climbing fiber axon is also localized (Sugihara and Shinoda 2007). Furthermore, this area topographically matches the termination area of axons of the Purkinje cells located in the area (or module) innervated by that climbing fiber axon (Sugihara et al. 2009). Therefore, projections of Purkinje cell axons and climbing fiber axon collaterals contribute to determining the compartmentalization of the cerebellar nuclei.

Mossy fiber axons do not necessarily have collaterals terminating in the cerebellar nucleus. Axons originating from the lateral reticular nucleus, nucleus reticularis tegmenti pontis, Clarke's nucleus, marginal Clarke's nucleus, and the ventral horn of the spinal cord often have collaterals terminating in the cerebellar nuclei, whereas axons originating from the external cuneate nucleus (Fig. 4g), pontine nucleus, and Stilling's nucleus rarely or infrequently have collaterals in the cerebellar nucleus (Gerrits and Voogd 1987; Wu et al. 1999; Quy et al. 2011; Luo et al. 2017, 2020; Biswas et al. 2019; Na et al. 2019; Zhang et al. 2021). A mossy fiber axon sometimes has multiple collaterals in different cerebellar nuclei or bilateral nuclei. Thus, the contribution of mossy fiber collaterals to the compartmentalization of the cerebellar nuclei seems various.

Serotoninergic and noradrenergic fibers densely innervate the cerebellar nuclei. Other axons have dense termination in the cerebellar nuclei but no termination (Luo and Sugihara 2014) or a trace of mossy fiber termination (Zhang et al. 2021) in the cerebellar cortex. Although these types of axons can effectively modify the output of the cerebellar nuclei neurons, they have not been much clarified.

5 Compartments or Modules of the Cerebellum

5.1 Cerebellar Modules Determined by Projections of Climbing Fibers and Purkinje Cell Axons

The topographic patterns of the projections of climbing fibers and Purkinje cell axons are tightly correlated (Ruigrok et al. 2015). Concerning this, a group of Purkinje cells located in similar mediolateral positions in different lobules convergently project to a common area within the cerebellar nucleus (Sugihara et al. 2009). Collaterals of olivary axons that project to this group of Purkinje cells then project to the same area in the cerebellar nucleus (Sugihara and Shinoda 2007). Furthermore, GABAergic neurons in this area of the cerebellar nucleus project back to the localized area in the inferior olive where those olivary axons originate (Ruigrok and Voogd 1990). As a whole, olivary axons and Purkinje cell projections are organized into longitudinal bands in the cerebellar cortex, which is the anatomical entity of the olivocorticonuclear "microcomplexes" (Ito 2012). Note that there are exceptions to the above basic organization scheme. For example, some Purkinje cell axons project to structures outside the cerebellar nucleus, such as the medial parabrachial nucleus

(Hashimoto et al. 2018). Some olivary axons branch transversely to project to Purkinje cells located in mediolaterally separate stripes (Fujita and Sugihara 2013).

The longitudinal bands described above are classified into several discreet groups (or "modules" as designated later: Ruigrok et al. 2015) that have a clear topographical relationship between subareas of the inferior olive and cerebellar nuclei (Voogd and Bigaré 1980; Buisseret-Delmas and Angaut 1993). At the broadest level, five gross modules (A, B, C1/C3, C2, and D modules) have been defined in the vermis, lateral vermis, medial and lateral paravermis, intermediate paravermis, and hemisphere, respectively, each with a topographically connected subarea in the IO and CN plus the lateral vestibular nucleus (Ruigrok et al. 2015). Finer classification of the modules has been identified (A, AX, X, X–CX, B, A2, C1/C3, CX, C2, D0, D1, and D2 modules: Buisseret-Delmas and Angaut 1993). The spatial distribution pattern of these modules in the cerebellar cortex matches the zebrin-striped pattern (see the next section) in the cerebellar cortex as reported in the rat and mouse (Voogd et al. 2003; Sugihara and Shinoda 2004; Sugihara and Quy 2007; Fig. 5c–e). In other words, each zebrin stripe has distinct topographical olivocerebellar and corticonuclear projection patterns (Voogd et al. 2003; Sugihara and Shinoda 2004; Sugihara et al. 2009; Fujita et al. 2010). A similar organization has been reported in the marmoset (Fujita et al. 2010). Inputs to subareas of the inferior olive are directly related to the functional significance of the cerebellar modules. They are described in Sect. 7 to some extent.

Besides the longitudinally striped organization described above, the lobular organization has also been observed in the olivocerebellar projection. In the vermis, generally distinct groups of climbing fiber axons that branch into (1) lobules I–V (or VIa) and VIII, (2) lobule IX, and (3) lobules (VIa), VIb–c, and VII have been observed (rat: Sugihara et al. 2001; Sugihara and Shinoda 2004; mouse: Sugihara and Quy 2007). In the hemisphere, a generally distinct group of climbing fiber axons project to (1) crus I and paraflocculus, and (2) simple lobule, crus II, and paramedian lobule. However, the lobular organization of the olivocerebellar and corticonuclear projections has not been fully clarified yet.

5.2 Longitudinal Stripes of Molecular Expression in the Cerebellar Cortex

Beyond the three classical longitudinal subdivisions (vermis, paravermis, and hemisphere), finer longitudinal subdivisions of the cerebellar cortex have been revealed. Purkinje cells are composed of heterogeneous populations of different molecular expression profiles. Aldolase C or zebrin II is the gold standard of such molecules in rat and mouse (Brochu et al. 1990; Voogd and Glickstein 1998; Sugihara et al. 2004; Fujita et al. 2014; Fig. 3b). The striped distribution patterns of aldolase C (zebrin II)-positive and aldolase C-negative Purkinje cells (zebrin pattern) have been clarified in the entire cerebellar cortex in the rat and mouse (Hawkes and

Leclerc 1987) as mapped in the unfolded scheme of the cerebellar cortex (rat: Sugihara and Shinoda 2004; Ruigrok et al. 2015; Sugihara and Quy 2007; mouse: Fujita et al. 2014; Sarpong et al. 2018; Fig. 2c). The striped distribution pattern is well conserved among individuals (Hawkes and Leclerc 1987; Fujita et al. 2014). Whereas the patterns in the rat and mouse have high similarity (Sugihara and Quy 2007; Fujita et al. 2014), the zebrin pattern in the marmoset, the sole primate species in which the zebrin pattern was clarified in the entire cerebellar cortex, shows basic characteristics shared with that of the rat and mouse as well as some unique characteristics (Fujita et al. 2010; Fig. 2b). Furthermore, all examined mammals seem to have more or less similar zebrin patterns at least in the anterior lobules (lobules I–V), where the three clear narrow positive stripes facilitate recognition (Sillitoe et al. 2005). The zebrin pattern is highly linked with the topographic connections of Purkinje cell axons and climbing fiber axons (see the preceding section).

The zebrin pattern is uniquely correlated with the lobular organization; the zebrin-striped patterns in different lobules are quite distinct from one another (Fujita et al. 2014). Simply, the cerebellar cortex is roughly divided into four areas ("zones" of Ozol et al. 1999) with distinct patterns of zebrin stripes in the rat and mouse. In lobules I–V and the rostral part of lobule VIa (anterior zone of Ozol et al. 1999), zebrin-negative stripes are much wider than zebrin-positive stripes. In vermal lobule VI (posterior part) and lobule VII, zebrin-positive stripes are much wider and occupy most areas (central zone). In lobule VIII, the anterior part of IX, crus II, paramedian lobule, and copula pyramidis (posterior zone), both zebrin-positive and zebrin-negative stripes occupy substantial areas. In the posterior part of lobule IX, X, paraflocculus, and flocculus, most areas are occupied by zebrin-positive Purkinje cells (nodular zone). However, if details of the zebrin pattern are compared in the entire cerebellar cortex, the area-dependent change in the pattern is more complicated than the above classification into four types, in the hemisphere in particular. One of the remarkable features of the striped pattern in the hemisphere is that all stripes shift laterally, and positive stripes are wider and merge in crus I in the rat and mouse (Sugihara and Shinoda 2004; Sugihara and Quy 2007) and in crus I and crus II, which are equivalent to rodent crus I (Luo et al. 2017), in marmoset.

Several other molecules are also expressed heterogeneously in Purkinje cell populations similar to aldolase C (zebrin). A group of molecules (e.g., EAAT4, PLCb3) shows nearly the same distribution as aldolase C. Another group of molecules such as PLCb4 shows the pattern precisely complementary to aldolase C (Sarna et al. 2006). Expression patterns of other molecules are different from the zebrin pattern to various degrees. For example, HSP25 is expressed in multiple longitudinal stripes in lobule VII, IX, paraflocculus, and flocculus in the mouse (Fujita et al. 2014). Tyrosine hydroxylase is expressed mainly in parts of zebrin-positive stripes in lobules VII–X and copula pyramidis, paraflocculus, and flocculus (Locke et al. 2020). Pcdh10 is expressed mainly in parts of aldolase C (zebrin)-positive stripes (Sarpong et al. 2018). Heterogeneous expression of molecules is presumably involved in (1) clustering of a population of neurons and formation of topographic afferent and efferent axonal projections in the embryonic period in the case of cell adhesion molecules such as EphA4 and Pcdh10 (Fujita et al. 2012; Vibulyaseck et al. 2017;

Tran-Anh et al. 2020), and (2) forming different synaptic responses, excitability and plasticity in the case of synaptic and signaling molecules such as EAAT4 and PLCb4 (Nguyen-Minh et al. 2019; Viet et al. 2021).

5.3 Compartmentalization of the Cerebellar Nuclei

The topographic projection patterns of Purkinje cell axons and collaterals of olivocerebellar climbing fiber axons define the functional compartmentalization of the cerebellar nuclei (preceding section). Thus, the cerebellar nuclei have subdivisions that approximately correspond to the longitudinally striped organization of the cerebellar cortex (Sugihara and Shinoda 2004, 2007). However, the positional correspondence between the nuclear subdivisions and cortical stripes is not simple. In the medial nucleus (MN), Purkinje cell axons from zebrin-positive stripes terminate in the ventral (in the most medial part of the MN) or caudoventral (in the rest of the MN) part of the medial nucleus. In contrast, Purkinje cell axons from zebrin-negative stripes terminate in the dorsal or rostrodorsal parts.

Consequently, the medial nucleus has caudoventral zebrin-positive and rostrodorsal zebrin-negative subdivisions because no other neurons other than Purkinje cell axons express zebrin in the cerebellar nuclei. The boundary between the zebrin-positive and zebrin-negative areas is not as clear as in the cerebellar cortex since the projection of Purkinje cell axons shows some spread beyond the boundary more or less. In the interpositus nucleus, zebrin-negative (and faintly-positive) Purkinje cell axons project to the rostrodorsal part, i.e., the anterior interpositus nucleus, and to the medial part of the posterior interpositus nucleus. In contrast, zebrin-positive Purkinje cells project to the ventral and lateral parts of the posterior interpositus nucleus. Thus, the striped arrangement of zebrin-positive and zebrin-negative stripes in the cerebellar cortex is transformed into the rostrodorsal versus caudoventral segregation of zebrin-positive and zebrin-negative areas in the medial and interpositus cerebellar nuclei (Sugihara and Shinoda 2007).

Furthermore, another type of subdivision in the cerebellar nuclei seems to be connected to different lobules. For example, in the lateral part of the posterior interpositus nucleus, Purkinje cells in crus II and simple lobules project dorsally while those in crus I and paraflocculus project ventrally (Luo et al. 2017; preliminary results). A similar tendency is present in the lateral nucleus (preliminary results of Owusu-Mensah et al.).

6 Output Projections of the Cerebellum

Most of the output projection of the cerebellum originates from cerebellar nucleus neurons. In addition, some Purkinje cell axons project to the targets outside the cerebellum. Although the number of output axons of the cerebellum is much smaller

than its afferent axons, the output axons are essential in conveying the integrated output signal of the cerebellum to other parts of the brain. A single output axon of the cerebellar nuclei generally has multiple targets by putative branching to be involved in diverse functions (Fujita et al. 2020). Transsynaptic labeling has revealed that the cerebellar output targets from any single lobule are remarkably diverse, covering multiple cerebral cortical areas and other forebrain areas (Pisano et al. 2021). Nonetheless, output projections of the cerebellar nuclei can be classified, based on the difference in the position, molecular expression profile, the projection pattern, and the main function involved as revealed in the medial nucleus (Fujita et al. 2020).

6.1 Somatomotor System

A classical view of the cerebellar somatomotor output system is that the medial nucleus mainly projects to the reticular formation and vestibular nucleus. These structures then give rise to reticulospinal and vestibulospinal descending projections, which compose the ventromedial subcortical descending motor system of the spinal cord (Lemon et al. 2012; Kuypers et al. 1962) to control the musculature of the neck, trunk, and proximal limb (extensors and flexors) for locomotion, antigravity movements, and posture. On the other hand, interpositus and lateral nuclei mainly project to the motor cortex through the ventrolateral thalamic nucleus and to the red nucleus. These structures then give rise to corticospinal and rubrospinal descending projections, which compose the cortical and lateral subcortical descending motor systems of the spinal cord (Lemon et al. 2012; Kuypers et al. 1962), respectively. These systems mainly control fine movements of extremities such as reach and grasp (Lemon et al. 2012).

In studies in rodents, the rostral part of the medial nucleus, which receives Purkinje cell projection from zebrin-negative stripes in the vermal lobules I–VIa and VIII, is the main source for the ventromedial motor system (Fujita et al. 2020). Additionally, Purkinje cells in the lateral vermis (zebrin stripes 2-) in lobules II–VIa project to the lateral vestibular nucleus (Deiters nucleus) (Sugihara et al. 2009), which is the origin of the lateral vestibulospinal projection. This projection also belongs to the ventromedial motor system (Kuypers et al. 1962) and is involved in controlling the flexor and extensor muscles of the limb.

The anterior interpositus nucleus, which receives Purkinje cell innervation from most of the zebrin-negative stripes in the paravermal and hemispheric areas, projects mainly to the red nucleus and ventrolateral thalamic nucleus (Teune et al. 2000). Thus, the output system of the anterior interpositus nucleus is involved in the cortical/rubral motor system. A well-known example is the control of the eyeblink conditioning performed in the zebrin-negative areas of the lateral paravermis and hemisphere in the junction between lobules V and VI, the lateral part of the anterior interpositus nucleus, and the red nucleus (Mauk et al. 2014). However, it would be worth mentioning that the anterior interpositus nucleus neurons have diverse

targets, besides the red nucleus and ventrolateral thalamic nucleus, such as other thalamic areas, zona incerta, several midbrain, pontine, and medullary areas (Teune et al. 2000), and the spinal cord down to the lumbar segments (Sathyamurthy et al. 2020). Neurons in the dorsal part of the lateral nucleus also project to the ventrolateral thalamic nucleus (Teune et al. 2000). After being relayed by the ventrolateral thalamic nucleus, the cerebellar output pathway reaches the motor and premotor cortices, where it meets the output of the basal ganglia relayed in the ventral lateral region of the thalamus adjacent to the cerebellar-recipient ventrolateral thalamic nucleus.

Some outputs from the caudoventral part of the medial nucleus and the lateral nucleus are relayed by the ventromedial and ventral anterior thalamic nucleus and project to the anterolateral motor cortex in rodents (Fujita et al. 2020; Gao et al. 2018; Chabrol et al. 2019), which is equivalent to the premotor area in primates. This pathway is involved in the preparatory activity of movements.

Some outputs of the caudodorsal part of the medial nucleus project to the intralaminar thalamic nuclei, such as the mediodorsal, parafascicular, and centrolateral nuclei (Fujita et al. 2020). These thalamic nuclei project to the striatum (Chen et al. 2014) and subthalamic nucleus (mainly through the parafascicular nucleus; Watson et al. 2021; Pisano et al. 2021). This is the pathway that the cerebellum can interact with the function of the basal ganglia such as selection, initiation, and learning of somatomotor and other behaviors, and generation of involuntary movements. The parafascicular nucleus also projects to the hippocampus and the amygdala (Kang et al. 2021).

6.2 Oculomotor System

A significant portion of the cerebellar output is involved in the control of the oculomotor system. Primarily, the final output of the oculomotor system is mediated mainly by six extraocular muscles innervated by three oculomotor nuclei in the brain stem. However, eye movements are controlled in multiple distinct ways, including vestibuloocular reflex (VOR), optokinetic reflex (OKR), vergence, saccade, smooth pursuit, and fixation. Different areas in the cerebellum are involved in the control of these different types of eye movements. The flocculus is involved in OKR and VOR, whereas the nodulus is involved in VOR. Purkinje cells in the flocculus and nodulus project not to the cerebellar nuclei but directly to the vestibular nuclei. The ventral part of paraflocculus is involved in the smooth pursuit in primates (Shidara and Kawano 1993), presumably relayed by the ventral part of the lateral nucleus. The caudal part of vermal lobules VI and VII ("oculomotor vermis") is involved in vergence, saccade, and smooth pursuit, relayed by the caudal part of the medial nucleus (Fujita et al. 2020). Besides the caudal part of the medial nucleus, the ventrolateral part of the posterior interpositus nucleus is also the source of the projection to the superior colliculus (Kawamura et al. 1982), possibly involved in saccades and orienting movements. This area of the nucleus is mainly innervated by the lateral part of lobule IX (stripe 4+; Sugihara et al. 2009).

6.3 Various Non-Motor Output Pathways

The output connection of the cerebellar medial nucleus to non-motor midbrain and pontine nuclei (ventral tegmental area, interpeduncular area, periaqueductal gray, and locus coeruleus) was first reported in a cat lesioning study (Snider and Maiti 1976). Subsequent studies have demonstrated other non-motor output projections, as discussed in the following paragraphs.

The ventral part of the lateral nucleus projects to the mediodorsal thalamus, which then projects to the prefrontal cortex (Middleton and Strick 2001). The prefrontal cortex is involved in various cognitive functions, emotional control, and motivation. The ventral part of the lateral nucleus is mainly innervated by the hemispheric lobules crus I and II in primates.

The most ventral part of the medial nucleus innervated by vermal zebrin-positive stripes 1+ and 2+//3+ projects to the parabrachial nucleus, Kölliker-Fuse nucleus, and inferior and medial vestibular nuclei (Fujita et al. 2020). Some Purkinje cells in the lateral vermis in lobules VIII and IX directly project to the parabrachial nucleus (Hashimoto et al. 2018). These output projections may be involved in the autonomic and visceral functions.

Some neurons in the medial, interpositus, and lateral nuclei project to the hypothalamus (Wen et al. 2004). The hypothalamus in return projects to the cerebellum through the pontine nucleus. This projection is involved in feeding, stress, and immune responses.

Other targets of the cerebellar output include the following. Dopaminergic and GABAergic neurons in the ventral tegmental area (Snider and Maiti 1976; Carta et al. 2019; Baek et al. 2022), which project to the medial prefrontal cortex and other areas (Kang et al. 2021), receive projections mainly from the lateral nucleus and additionally from the posterior interpositus and medial nuclei. The periaqueductal gray (Frontera et al. 2020; Vaaga et al. 2020) receives projections mainly from the lateral and medial nuclei. Serotoninergic neurons in the dorsal raphe nucleus receive projections from the lateral nucleus (Pollak Dorocic et al. 2014). Noradrenergic and GABAergic neurons in the locus coeruleus are innervated by the cerebellar nuclei and some vermal Purkinje cells (Schwarz et al. 2015). Some cerebellar nucleus neurons also project to the zona incerta, substantia nigra, laterodorsal tegmental nucleus, pedunculopontine tegmental nucleus, nucleus incertus, and supramammillary region (Teune et al. 2000; Fujita et al. 2020; Kebschull et al. 2020; Kang et al. 2021).

6.4 Inhibitory Output Projection from the Cerebellar Nuclei

Small GABAergic neurons present in all deep cerebellar nuclei send their axons to the contralateral inferior olive through the superior cerebellar peduncle. The topographic relationship of this projection matches the topography of the olivonuclear

and olivocorticonuclear projections (Ruigrok and Voogd 1990). This inhibitory projection terminates mainly in the dendritic region, which forms dendrodendritic gap junctions in inferior olive neurons, affecting electrical coupling between neurons.

The medial nucleus contains many glycinergic neurons that project to the vestibular and reticular formation in the ipsilateral brain stem (Bagnall et al. 2009), presumably constituting a part of the ventromedial somatomotor system (*see* Sect. 6.1).

7 Functional Localization in the Cerebellum

Animal recording, lesioning, and genetically manipulating studies, and human imaging studies have revealed functional localization (Brodal 1981; Schmahmann et al. 2019). Topography of afferent and efferent axonal projection patterns underlies the cerebellar functional localization. The functional localization is usually described based on clear landmarks of the cerebellum, i.e., lobules and the distinction between the vermis and the hemisphere. The longitudinally striped patterns such as zebrin stripes and A–D modules (Fig. 5c) must also be considered. Indeed, climbing fiber activity in different stripes occurs in a different context in crus II of behaving mice (Tsutsumi et al. 2019). However, zebrin stripes or A–D modules have not been identified in most functional studies, or in any primate or human studies. Therefore, in this section, the lobular organization, which is mainly linked with mossy fiber projection patterns (Fig. 5a), is focused primarily. Striped or modular subdivisions (Fig. 5c) are mentioned secondly within each lobular division.

It is also to be noted that multiple areas located in different functional localization domains can simultaneously control different aspects of a single motor behavior as in the case of involvement of vermis-medial nucleus in eyeblink conditioning (Wang et al. 2020).

7.1 Functional Localization of Vermal Areas

7.1.1 Lobules I–VIa and VIII

Vermal lobules I–VIa and VIII (Fig. 6a, brown) are involved in the control of locomotion and posture (Jahn et al. 2008; Coffman et al. 2011; Ozden et al. 2012; Luo et al. 2020). These lobules receive information mainly related to limb, trunk, and neck deep and cutaneous sensations through mossy fiber projections from the spinal cord (Luo et al. 2017, 2020; Zhang et al. 2021), dorsal column nuclei (Quy et al. 2011), and lateral reticular nucleus. These lobules also receive mossy fiber projections from the caudal part of the pontine nucleus that mediates signals mainly from hindlimb somatotopic areas of the somatosensorimotor cortices (Biswas et al. 2019;

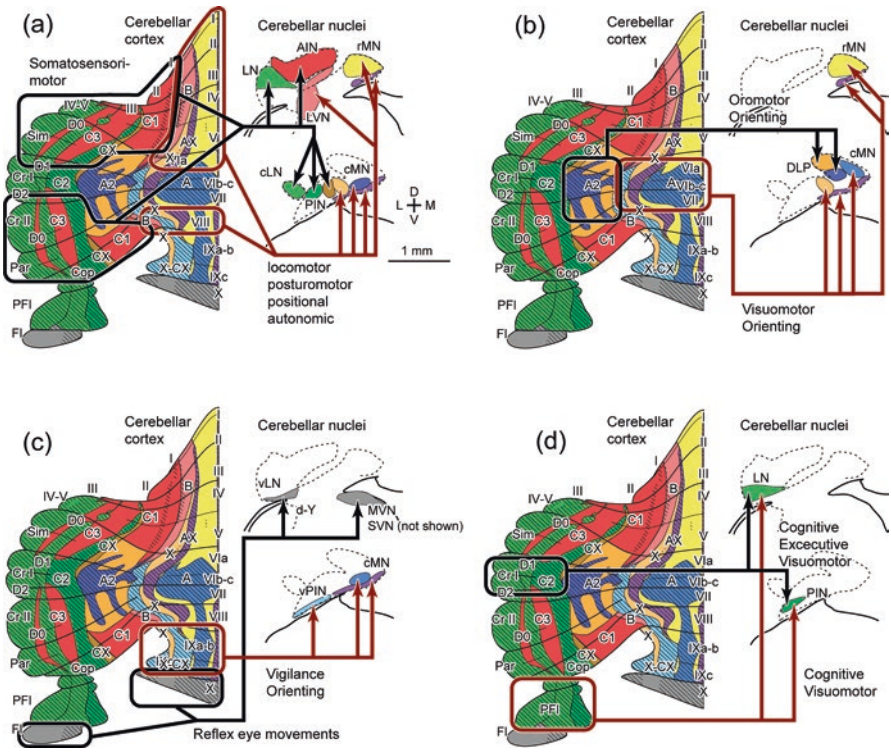

Fig. 6 Topographic projection patterns of Purkinje cell axons linked with the functional localization of the cerebellar cortex. (**a**–**d**) The whole cerebellar cortex was divided into eight areas of major functional localization. The topographic projection patterns from these areas to subareas of the cerebellar nuclei are summarized (black and brown circumscribed areas and arrows). In each panel, the zebrin pattern (shaded) and divisions of topographic olivocerebellar climbing fiber projections and corticonuclear Purkinje cell projections of the mouse cerebellar cortex are shown on the left side. On the right side, rostral (top) and caudal (bottom) levels of the mouse cerebellar nuclei are drawn. Target areas of the projection are mapped in the same color as the origin in the cortex. The proposed function for each division is indicated by the arrow. The scheme is based on Sugihara and Shinoda (2004, 2007), Sugihara et al. (2009), Sarpong et al. (2018), Fujita et al. (2020), and preliminary data by Owusu Mensah, Luo Yuanjun, and Izumi Sugihara. Abbreviations: I–X, lobules I–X; a–c, sublobules or subareas a–c; AIN, anterior interposed nucleus; cLN, caudal part of the lateral nucleus; cMN, caudal part of the medial nucleus; Cop, copular pyramids; Cr I, crus I; Cr II, crus II; D, dorsal; DLP, dorsolateral protuberance of the medial nucleus; d-Y, dorsal Y nucleus; Fl, flocculus; L, lateral; LN, lateral nucleus; LVN, lateral vestibular nucleus; M, medial; MN, medial nucleus; MVN, medial vestibular nucleus; Par, paramedian lobule; PFl, paraflocculus; Sim, simple lobule; PIN, posterior interposed nucleus; SVN, superior vestibular nucleus; V, ventral; vLN, vPIN, ventral part of the LN, PIN

Coffman et al. 2011). There are some lobule-dependent and stripe-dependent differences in mossy fiber projections in these lobules. Zebrin-negative stripes are much wider than zebrin-positive stripes in these areas. Climbing fibers in these lobules mainly arise from the central and lateral parts of the caudal part of the medial accessory olive (cMAO-b and cMAO-a). The output of these areas is relayed by the

rostral and ventral parts of the medial nucleus and projects to the brain stem reticular formation and other areas (group F1R -“posturomotor”- and F3 -“positional-autonomic”- of Fujita et al. 2020).

The most lateral part (zebrin stripe 2-//4-) of these vermal areas has a special output connection. Purkinje cells in this part project directly to the lateral vestibular nucleus (Deiters’ nucleus), where large neurons give rise to the lateral vestibulospinal tract to control ipsilateral anti-gravity muscles.

7.1.2 Lobules VIb–c and VII

Vermal lobules VIb–c and VII (Fig. 6b, brown) are involved in the non-motor function and oculomotor control (Suzuki et al. 2012; Watson et al. 2014; Catz and Thier 2007). Human imaging studies show cognitive function in vermal lobules VI and VII (Guell et al. 2018). These areas receive mossy fiber projections mainly from the rostral, medial, and lateral parts of the pontine nucleus, which relay the signals from association cortices, including the medial prefrontal cortex (Biswas et al. 2019). Zebrin-positive stripes occupy most of these areas. Climbing fibers in these areas mainly arise from the medial parts of the caudal part of the medial accessory olive (cMAO-c). Many targets in the pons, midbrain, and thalamus receive relay information from these areas through the caudodorsal parts of the medial nucleus (group F2-“orienting” of Fujita et al. 2020).

7.1.3 Lobule IXa–b

Lobule IXa–b (Fig. 6c, brown) is involved in functions related to the orientation and sensory processing of the face and head (Waespe et al. 1985; Welker 1987; Sugihara 2005). These areas receive mossy fiber projections mainly from the external cuneate nucleus (Quy et al. 2011) and trigeminal nucleus (Welker 1987) and additionally from the pontine nucleus and spinal cord (Luo et al. 2017; Zhang et al. 2021). Zebrin-positive stripes predominate these areas. Climbing fibers in these areas mainly arise from the subnucleus beta (to medial parts of lobule IXa–b), and the caudal part of the ventral lamella of the principal olive (Sugihara and Shinoda 2004). The output of the medial parts of this lobule is relayed by the caudoventral parts of the medial nucleus and projects to many targets in the medulla, pons, midbrain, and thalamus (group F4-“vigilance” and F3 -“positional-autonomic”- of Fujita et al. 2020). The output of the lateral parts of this lobule innervates the lateral ventral part of the posterior interpositus nucleus (Sugihara et al. 2009), which then projects to the superior colliculus (Kawamura et al. 1982) and other unidentified targets.

7.1.4 Lobules IXc and X

Lobules IXc (ventral uvula) and X (nodulus) (Fig. 6b, black) are involved in the control of adaptation of vestibuloocular reflex and vestibular reflexes of head and body orientation and in motion sickness (Barmack et al. 1992; Liu and Angelaki 2009; Cohen et al. 2019). These areas receive mossy fiber projections mainly from the vestibular nucleus (Ando et al. 2020) and project output directly to the vestibular nuclei. Zebrin-positive stripes occupy almost all of these areas. Climbing fibers in these areas mainly arise from the dorsal cap and ventrolateral outgrowth (Sugihara et al. 2004).

7.2 Functional Localization of Paravermal and Hemispheric Areas

7.2.1 Rostral and Caudal Lobules

The paravermal and hemispheric areas in lobules HIII–HV, HVI, HVIIB, and HVIII (in the human cerebellum; hemispheric lobules III–V, simple lobule, crus II, paramedian lobule, and copula pyramidis are equivalent lobules in the rodent cerebellum; Fig. 6a, black) are both involved in somatosensorimotor control of fine motor activity of body parts in a somatotopic manner (Thickbroom et al. 2003; Manni and Petrosini 2004; Tran-Anh et al. 2020; Fig. 5b). The body area is dually represented in the above two areas in the cerebellum. In the rostral part (lobules III–HVI), the hindlimb, forelimb, and face are represented in more mediorostral, intermediate, and laterocaudal areas, respectively. In the caudal part (lobules HVIIB and VIII), the hindlimb, forelimb, and face are represented in more mediocaudal, intermediate, and laterorostral areas, respectively. Thus, the arrangements of somatotopic representation in the rostral and caudal lobules are more or less in a mirror-image relationship. The somatotopy arrangement in these areas is not as clearly represented as in the cerebral cortex. These lobules receive mossy fiber projections mainly from the pontine nucleus (Biswas et al. 2019), trigeminal nucleus (Welker 1987), dorsal column nuclei, lateral reticular nucleus, and spinal cord in a somatotopic manner.

Zebrin-negative and zebrin-faintly positive stripes (equivalent to the C1/C3 module) occupy most of these areas in the paravermal and medial hemispheric parts. Climbing fibers in these areas mainly arise from the dorsal accessory olive (to medial and intermediate parts or the hindlimb and forelimb somatotopy areas) and the dorsomedial subnucleus (to the lateral parts or the face somatotopy area) (Sugihara and Shinoda 2004; Cerminara et al. 2013). The rostral and caudal lobules are topographically innervated by branching climbing fiber axons. The output is relayed mainly by the anterior interpositus nucleus, in a somatotopic manner (hindlimb, forelimb, and face, from the medial to the lateral parts). The rostral and caudal lobules project convergently to the anterior interpositus nucleus (Sugihara and Shinoda 2004; Sugihara et al. 2009). The anterior interpositus nucleus then projects mainly to the ventrolateral thalamic nucleus and the red nucleus. The

anterior interpositus nucleus and the cortical areas that are topographically connected to the interpositus nucleus are involved in the control of fine body movements such as grasping, limb cutaneous reflexes, and eyeblink reflex (Ekerot et al. 1997; Pijpers et al. 2008; Horn et al. 2010; Low et al. 2018).

Zebrin-positive stripes occupy only a limited extent in these areas (paravermal and hemispheric areas in lobules HIII–HV, HVI, HVIIB, and HVIII). A positive stripe (4+//5+ or C2 module) is present in the paravermal part, and two positive stripes (5+//6+ and 6+//7+ or D1 and D2 modules) are present in the lateral hemispheric parts. Climbing fibers in these areas mainly arise from the rostral part of the medial accessory olive and the principal olive (Sugihara and Shinoda 2004). The output is relayed by the dorsal parts of the posterior interpositus nucleus and the lateral nucleus (Luo et al. 2017; macrogyric part of the human dentate nucleus, Steele et al. 2017), and projects to the thalamus and other targets. How distinct is the function of these zebrin-positive areas from the function of zebrin-negative and zebrin-faintly positive stripes has not been much clarified.

7.2.2 Medial Paravermal Area of Lobules VI and VII (Lateral A Module)

The medial paravermal area of lobules VI and VII (Fig. 6b, black) has climbing fiber and Purkinje cell projections similar to those in the vermis, and is thus designated as the "lateral A module." This area is more prominent in the rodent cerebellum than in the primate cerebellum (Fujita et al. 2010), and is composed of alternating zebrin-positive and zebrin-negative stripes. Developmentally, this area is formed by the lateral migration of Purkinje cell clusters in the central part of the cerebellum (Vibulyaseck et al. 2017). The climbing fibers originate from the medial area of the caudal part of the medial accessory olive. The output of these areas is relayed by the dorsolateral protuberance of the medial nucleus and projects to many targets in the medulla, pons, midbrain, and thalamus (group F1rDLP-"oromotor" and a part of F2-"orienting" of Fujita et al. 2020).

7.2.3 Ansiform Area (Crus I in Rodents, Crus I + II in Primates)

Imaging studies in humans have shown that crus I and crus II (or the ansiform area, equivalent to only crus I in the rodent cerebellum) (Fig. 6d, black) are mainly involved in cognitive, executive, language processing, and saccadic functions (Stoodley and Schmahmann 2009; Batson et al. 2015; D'Mello and Stoodley 2015; Guell et al. 2018). Primate studies characterize crus I and crus II by their connectivity to the prefrontal cortex underlying non-motor functions (Strick et al. 2009). In rodents, manipulation of crus I elicits cognitive, execution, and autism-relevant responses (Stoodley et al. 2017; Kelly et al. 2020). These areas receive mossy fiber projections mainly from the rostral, medial, and lateral parts of the pontine nucleus (Biswas et al. 2019). Zebrin-positive stripes occupy most of these areas. Climbing fibers in these areas mainly arise from the medial parts of the caudal parts of the

medial accessory olive, rostral parts of the medial accessory olive, and rostrolateral parts of the principal olive (Sugihara and Shinoda 2004). These parts of the inferior olive receive projections from the mesodiencephalic junction that mediates the corticofugal projection (Wang et al. 2022). The output is relayed by the ventral parts of the posterior interpositus nucleus and lateral nucleus (Luo et al. 2017; microgyric part of the human dentate nucleus, Steele et al. 2017). The output then has diverse projections to various forebrain areas including the subthalamic nucleus and association cortices (Pisano et al. 2021).

7.2.4 Paraflocculus

Human imaging studies showed cognitive function in lobule HIX (e.g., Guell et al. 2018), which is equivalent to the paraflocculus (Fig. 6d, brown). Functional localization of the paraflocculus is not much clarified besides the control of smooth pursuit eye movements in primates (Shidara and Kawano 1993) and tinnitus in rats (Bauer et al. 2013). The possible involvement in non-motor function may be further studied in the animal paraflocculus. The paraflocculus has similar mossy fiber innervation as crus I; it mainly receives projections from the rostral, medial, and lateral parts of the pontine nucleus (Biswas et al. 2019; Na et al. 2019). The whole paraflocculus is zebrin-positive. Climbing fiber projection to this area often comes from branches of the climbing fiber axon projecting to crus I in the rat (Fujita and Sugihara 2013). Climbing fibers in these areas mainly arise from the rostral parts of the medial accessory olive, and rostrolateral parts of the principal olive (Sugihara and Shinoda 2004), which receives projections from the mesodiencephalic junction, which mediate corticofugal projection (Wang et al. 2022). The output is relayed by the posterior interpositus nucleus and dentate nucleus. The output then has diverse projections to various forebrain areas including the subthalamic nucleus and association cortices (Pisano et al. 2021). The overall axonal projection patterns in the paraflocculus have some similarities to those in the ansiform area (crus I in rodents) (Fujita and Sugihara 2013; Biswas et al. 2019).

7.2.5 Flocculus

The flocculus (Fig. 6c, black) is involved in the control of reflex eye movements such as vestibuloocular reflex and optokinetic reflex (monkey: Lisberger and Fuchs 1978; cat: Sato and Kawasaki 1984; rabbit: Ito et al. 1977; Barmack et al. 1992; mouse: Koekkoek et al. 1997). The mossy fiber projection to the flocculus mainly comes from the vestibular nuclei (Ando et al. 2020), the primary vestibular afferent, and the contralateral lateral and medial pontine nucleus. The flocculus is composed of three major longitudinally striped areas (Sugihara et al. 2004). The central stripe is involved in horizontal-directional eye movements and receives climbing fiber projections from the dorsal cap. The rostral and caudal stripes are involved in vertical-directional eye movements and receive climbing fiber projections from the

ventrolateral outgrowth. The dorsal cap and the ventrolateral outgrowth are neighboring mediodorsal small subnuclei of the inferior olive. These areas in the flocculus project to the most ventral part of the lateral nucleus, dorsal Y nucleus, and some areas in the medial and superior vestibular nuclei. The rostral half of the flocculus has weaker zebrin expression than the caudal half (Fujita et al. 2014), although matching of the zebrin expression pattern in the flocculus to the three functional longitudinally striped areas is unidentified yet.

8 Concluding Remarks

This chapter summarized the macroscopic and histological morphology, axonal connections, and functional localization of the mammalian cerebellum. Although the description is mostly based on findings in rodents, there is a fairly comparative analysis involving human and primate findings. The relationship between the lobular organization and longitudinally striped compartmentalization, and the organization of the olivocorticonuclear topographic connection is complicated. Therefore, we tried to draw a comprehensive general picture rather than making an extensively detailed description in this chapter. Further information is available in Baek et al. (2022) and Pisano et al. (2021) about output projections, Fujita et al. (2020) about the output organization of the medial nucleus, and Luo et al. (2017) and Sugihara (2021) about the ansiform area definition in rodents and primates.

Competing Interests The authors have no competing interests to declare.

Acknowledgments This chapter was supported by Grant-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (19K06919 and 21F21107 to I.S., and 19H21208 and 20K15911 to Y.L.). L.Y. is a recipient of the JSPS Postdoctoral Fellowship for Overseas Researchers. O.M.R.N.A. is a recipient of the MEXT Scholarship for Overseas Students.

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Cerebellar Physiology

Jasmine Pickford and Richard Apps

Abstract The cerebellum is typically associated with motor control although there is now extensive evidence that its involvement extends into other domains including cognitive processing. The cerebellum contains a highly regular neural organization, but exactly how this circuitry contributes to its diverse functions remains unclear. Patterns of inputs to and outputs from the cerebellum, together with intracerebellar connections, add layers of complexity to cerebellar computations that can differ between anatomically and physiologically defined modules. Different modules are therefore likely to be specialized for different functions, for example in balance and locomotion versus reach-to-grasp. However, the unifying role of the cerebellum in the control of motor and cognitive behavior may be to serve as a prediction device, refining these predictions based on actual outcomes, to enhance behavioral performance.

Keywords Cerebellum · Purkinje cell · Climbing fiber · Mossy fiber · Cerebellar nuclei · Module · Motor · Learning · Prediction

1 Introduction

This chapter aims to provide an overview of the physiology of cerebellar circuits as a framework for the consideration of other chapters in this book. The physiology of the cerebellum has been studied intensively for over a century. A step change in understanding occurred in the 1960s with the pioneering work of Eccles and colleagues, who electrophysiologically characterized the intricate neuronal circuitry of the cerebellum and thus provided a physiological foundation upon which many future studies were based (Eccles et al. 1967). Given the huge subject area, it is beyond the scope of this chapter to consider all aspects in detail. Instead, the aim is

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_2

to provide an up to date introduction for those unfamiliar to the topic (and those that welcome a refresher), with a focus on cerebellar physiology in relation to voluntary behavior. This provides the foundations for understanding the etiology of cerebellar disease including the focus of this book, namely ataxia, which is defined as the impaired coordination of voluntary muscle movement (Ashizawa and Xia 2016). The reader is directed to previous reviews for further details (e.g., Middleton and Strick 1998; Garwicz 2002; Llinás et al. 2002; Apps and Garwicz 2005; Ito 2006; Courtemanche et al. 2013; Jörntell 2017; D'Angelo 2018).

The current chapter will consider cerebellar physiology in the context of neural circuit loops, including olivo-cortico-nuclear connections, local cerebellar cortical circuits, and reciprocal patterns of input and output connectivity with other brain structures. As a consequence of these circuit loops, rhythmicity and synchronicity appear to be important physiological features of the cerebellum. Evidence is also accumulating to indicate that physiology is non-uniform across cerebellar regions, so caution should be made in assuming the same information transform occurs throughout the cerebellum.

The chapter will also explore how physiology translates to behavior, and evidence is presented that the cerebellum acts as a feedforward controller to modulate behavior. Generally speaking, the cerebellum is thought to contain internal models of effector systems that allow it to refine ongoing behaviors without waiting for sensory feedback (Wolpert et al. 1998). As such, the cerebellum is likely to control behavior by generating predictions about future behavioral outcomes that are updated based on the comparison of actual and expected outcomes (Hull 2020). Similar predictive mechanisms may apply across motor and cognitive domains, allowing the cerebellum to optimize the many types of behavior in which it is now known to be involved.

2 Basic Cerebellar Structure

2.1 Gross Cerebellar Structure

In order to understand cerebellar physiology, it is necessary to first consider cerebellar anatomical organization. Broadly speaking, the cerebellum has two major subdivisions: the cerebellar cortex and cerebellar nuclei (CN). The cerebellar cortex is highly convoluted and encapsulates the CN, which are situated deep within the cerebellum and are thus often referred to as the deep cerebellar nuclei. From medial to lateral, each half of the cerebellum can be classified into three longitudinal compartments—the vermis, paravermis (intermediate), and hemispheres—and across all compartments the cerebellar cortex has the same basic trilaminar structure, composed from inner to outermost by the granule cell layer, the Purkinje cell (PC) layer, and the molecular layer (Fig. 1).

The granule cell layer contains granule cells (the most numerous neuronal cell type in the nervous system), Golgi cells, unipolar brush cells (UBCs), Lugaro cells,

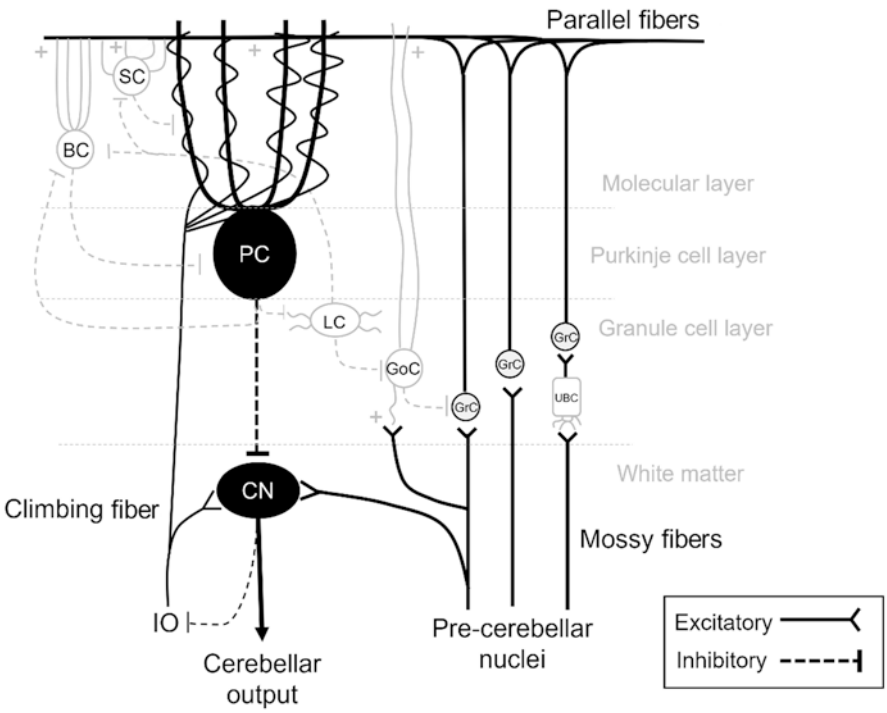

Fig. 1 Simplified cerebellar circuitry. Inputs to the cerebellum are mainly from mossy fibers and climbing fibers. Mossy fibers synapse onto granule cells (GrCs) that form bifurcating axons known as parallel fibers. Purkinje cells (PCs) receive inputs from parallel fibers and climbing fibers and their main target is the cerebellar nuclei (CN). Other local neurons are also present in the molecular and granule cell layers of the cerebellar cortex. Nucleo-cortical connections, candelabrum cells, and Bergmann glia are not shown. Abbreviations: BC basket cell, GoC Golgi cell, GrC granule cell, IO inferior olive, LC Lugaro cell, SC stellate cell, UBC unipolar brush cell

and a subgroup of Lugaro cells known as globular cells (Lainé and Axelrad 2002). The PC layer contains PCs—the only output neuron of the cerebellar cortex—and candelabrum cells (a type of interneuron: Lainé and Axelrad 1994), as well as Bergmann glia—astrocytes that contribute to cerebellar information processing (De Zeeuw and Hoogland 2015). Finally, the molecular layer contains interneurons including stellate cells and basket cells (Fig. 1). Granule cells and UBCs are excitatory, whereas all the other types of neurons in the cerebellar cortex are thought to be inhibitory.

In terms of cerebellar output there are three major subdivisions of the CN located in each half of the cerebellum, from medial to lateral: the medial (fastigial), interpositus (anterior and posterior divisions), and lateral (dentate) nuclei, which receive topographically organized cortico-nuclear inputs from PCs in the overlying vermis, paravermis, and hemispheral cortex, respectively (Voogd 1967; Voogd and Glickstein 1998).

2.2 *Cerebellar Inputs*

Climbing fibers and mossy fibers form the two major synaptic inputs to the cerebellum and both are excitatory (glutamatergic). In addition, there are several, far less studied, neuromodulatory inputs that project with varying patterns and densities throughout the cerebellum.

2.2.1 Basic Anatomy of Climbing Fiber Projections and Olivo-Cortico-Nuclear Circuits

Climbing fibers originate from a single brainstem nucleus, the inferior olive, which in turn receives widespread inputs from the spinal cord, brainstem, CN, and higher centers including the motor cortex (Llinas et al. 2004). Climbing fibers make direct synaptic contact with cerebellar cortical PCs, and the physiological consequences of this intimate connectivity are discussed later in Sect. 3.1. Several climbing fibers originate from each inferior olive neuron (on average approximately seven per neuron in rats), but each PC is only innervated by a single climbing fiber in the adult rat (Sugihara et al. 1999). The stem axon of individual olive neurons therefore branches to provide climbing fiber input to multiple PCs that are arranged mainly in the rostrocaudal axis (Apps and Garwicz 2005). On their path to the cerebellar cortex, olivary axons also form collateral inputs onto CN neurons (Fig. 1), typically forming between one and six collaterals terminating in a particular nucleus (Sugihara et al. 1999).

Small populations of neurons located within different subnuclei of the inferior olive give rise to climbing fibers that target a specific rostrocaudally orientated "zone" of PCs in the cerebellar cortex (Fig. 2, Apps and Garwicz 2005). These zones can be identified both anatomically and physiologically, with each zone typically one to three millimeters in mediolateral width but extending for many millimeters in the rostrocaudal axis (Apps and Hawkes 2009). The PCs in each cortical zone provide a convergent cortico-nuclear inhibitory projection to neurons in a specific region of the CN, thereby forming multiple, olivo-cortico-nuclear connections termed "modules" (Fig. 2, Apps and Garwicz 2005; Apps and Hawkes 2009).

This modular organization extends to nucleo-olivary projections arising from the same region of CN that provides inhibitory feedback to the originating olivary subnucleus via GABAergic projections (Fig. 2). As a result, PCs can influence their own climbing fiber inputs by modulating the inferior olive neurons from which the climbing fibers arise, via CN neurons (as shown in paravermal regions by Chaumont et al. 2013). Individual cerebellar modules are thought to subserve different functions (Horn et al. 2010, although see Cerminara and Apps 2011). For example, the vermal A module is associated with head movements, balance, and postural control; the paravermal C modules are involved in limb movements, including grasping; and the lateral D2 module is involved in predicting target motion during visually guided movements (Fig. 2c; Cerminara and Apps 2011). It is important, however, to

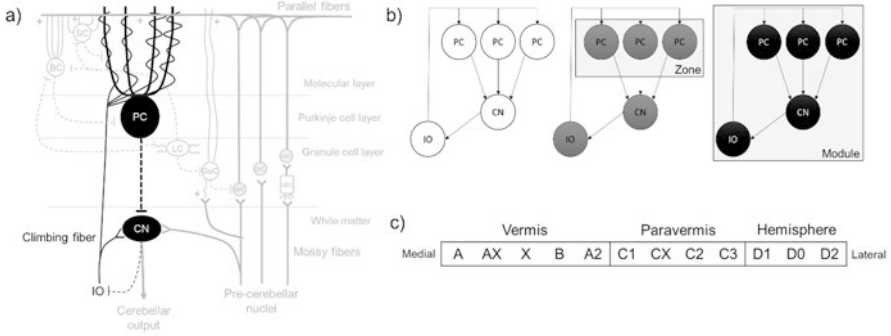

Fig. 2 Cerebellar zones and modules. (**a**) Olivo-cortico-nuclear circuit within the cerebellar circuitry (see Fig. 1 for abbreviations); (**b**) Purkinje cells (PCs) receiving common inferior olive (IO) climbing fiber inputs form a "zone," and these PCs together with their IO input and cerebellar nuclei (CN) output regions form a "module"; (**c**) Modules defined in rats in each half of the cerebellum from medial to lateral

emphasize that individual modules are not likely to be restricted to specific functions, not least because of the various interactions that can occur between them (see below).

The cortical component of some modules can be further divided into microzones that contain PCs with similar climbing fiber receptive fields (e.g., Andersson and Oscarsson 1978; Ekerot et al. 1991). Microzones and their microcomplex connections with the CN and inferior olive are thought to represent the basic functional units of the cerebellum (Apps and Hawkes 2009).

2.2.2 Basic Anatomy of Mossy Fiber Projections

In stark contrast to the climbing fiber system, mossy fibers arise from multiple brain nuclei distributed throughout the central nervous system, including all segmental levels of the spinal cord, numerous brainstem nuclei, but most notably the basilar pontine nuclei (which receive inputs primarily from the neocortex; Llinas et al. 2004). Mossy fibers branch widely in the cerebellar cortex, usually in the rostrocaudal dimension, and in the rat follow a similar pattern of termination as climbing fibers in the overlying molecular layer, although their organization is less precise (Voogd et al. 2003; Pijpers et al. 2006). This suggests that broadly speaking, mossy fiber termination patterns adhere to the modular organization of the olivo-cortico-nuclear system, and that these projections are targeted to certain functions rather than forming a diffuse, generalized input. However, given that mossy fibers have collaterals in the mediolateral plane they may be able to influence multiple modules (Shinoda et al. 1992; Wu et al. 1999), and mossy fiber projections may also vary within a given module as demonstrated in the C1 zone of the rat (Herrero et al. 2002, 2012).

Mossy fibers synapse onto granule cells in the granule cell layer and many also form excitatory collateral inputs to CN neurons on their route to the cortex (Fig. 1). Mossy fiber collaterals arising from a given axon may target different divisions of the CN, again suggesting that mossy fiber inputs are not universally aligned with climbing fiber inputs to the cerebellum (Wu et al. 1999). Granule cell axons bifurcate to form parallel fibers that contact many PCs in the long axis of individual cerebellar folia and so multiple cerebellar modules can be connected via common parallel fiber inputs (Valera et al. 2016; Binda et al. 2016).

A subset of mossy fibers (approximately 5% in cat) arise from the CN and provide a nucleo-cortical feedback projection to the cerebellar cortex, targeting the granule cell layer (Houck and Person 2014). While some of these neurons target areas of the cerebellar cortex that provide reciprocal PC cortico-nuclear projections, others target regions of the cortex from which they receive no input, thereby forming an additional route for modules to interconnect (Trott et al. 1998a, b). In mice, a proportion of the nucleo-cortical connections arise from collaterals of the large glutamatergic projection neurons in the CN (Houck and Person 2015) and are thought to act as an internal amplification system to assist associative learning (Gao et al. 2016). In addition, a subpopulation of nucleo-cortical neurons, also described in mice, are inhibitory and target Golgi cells in the granule cell layer, thereby allowing disinhibition of cerebellar cortical circuits (Ankri et al. 2015).

2.2.3 Neuromodulatory Inputs

As well as climbing fiber and mossy fiber glutamatergic inputs, neuromodulatory afferents targeting the cerebellum release either serotonin, noradrenaline, acetylcholine, dopamine, or histamine (Schweighofer et al. 2004). These inputs differ in their pattern of termination and are not uniformly distributed throughout the cerebellum. Far less is known about their physiology but they may have roles in regulating cerebellar development (Oostland and van Hooft 2013), synaptic transmission and plasticity (Lippiello et al. 2015), and modifying cerebellar activity throughout different stages of the sleep–wake cycle (Brown et al. 2001; Jaarsma et al. 1997).

2.3 Non-uniformity in Cerebellar Anatomy

The preceding sections outline the classical, orderly microcircuit organization of the cerebellum, characterized by olivo-cortico-nuclear loops. However, it is becoming increasingly clear that there are also important regional variations in anatomy that confer differences in physiological properties. In particular, it has long been known that a variety of molecular markers are differentially expressed throughout the cerebellar cortex, providing anatomical and physiological subdivisions. Most notable among these markers is zebrin II (also known as aldolase C), which in some regions of the cerebellar cortex is expressed in subsets of PCs forming a highly

characteristic and reproducible pattern of stripes, with alternating positive and negative rostrocaudally oriented bands of expression.

Zebrin II colocalizes with several other markers, such as phospholipase Cβ3, excitatory amino acid transporter 4 (EAAT4), and metabotropic glutamate receptor 1a (mGluR1a), while some markers are present only in zebrin II-negative PCs, such as phospholipase Cβ4. This pattern of protein distribution appears to be present in the cerebellum of all birds and mammals, including humans, and in some regions of the cerebellar cortex has been found to correspond to the organization of both mossy fiber and climbing fiber inputs (Apps and Hawkes 2009). This relationship between molecular signature and anatomical circuits extends to PC cortico-nuclear projections, suggesting a common spatial organization of cerebellar cortical inputs, PC phenotype, and cortico-nuclear outputs (Apps and Hawkes 2009).

Another important example of non-uniformity is the distribution of UBC cerebellar cortical interneurons. These are glutamatergic, located in the granule cell layer (Fig. 1), and are found mainly in the vermis and flocculonodular lobe where they provide feedforward amplification of cerebellar inputs. Since these regions of the cerebellum are known to be involved in the regulation of body, head, and eye position, UBCs are thought to serve a specific cellular function within these cerebellar regions relating to these behaviors (Mugnaini et al. 2011). In mice, PC collaterals to UBCs preferentially inhibit UBCs expressing metabotropic glutamate receptor 1 (mGluR1), adding an extra level of heterogeneity even within regions containing UBCs (Guo et al. 2021).

3 Cellular Physiology

3.1 Cortical Circuits

3.1.1 Inputs to the Granule Cell Layer

Granule cells account for over half of all neurons in the human brain (Herculano-Houzel 2010). Their primary input is from mossy fibers, which terminate in structures called glomeruli, with an average of four glutamatergic mossy fiber inputs to each granule cell (Eccles et al. 1967). The structure of a glomerulus allows glutamate released from one mossy fiber terminal to spillover onto neighboring granule cell dendrites within the glomerulus, which may improve efficacy of neurotransmission (DiGregorio et al. 2002). Transmission at mossy fiber–granule cell synapses is thought to be highly secure, with stimulation of a single mossy fiber at high frequencies evoking granule cell firing in vivo (Rancz et al. 2007). Other studies, however, suggest that synchronous input from multiple mossy fibers is required to evoke granule cell firing, and that subthreshold signals are filtered out (Jörntell and Ekerot 2006). These differences in synaptic efficacy may be the result of a number of possibilities, including regional variations (see Sect. 2.3) and the nature of the inputs being encoded.

Mossy fibers transmit sensorimotor, proprioceptive, and contextual information, with inputs from different body parts represented in different cerebellar regions in line with the somatotopic organization of the cerebellum (see Sect. 4.1; Arenz et al. 2009). Granule cells may receive input from multiple modalities; for example vestibular, visual, and eye-movement-related signals converge on individual granule cells in the flocculus of mice (Arenz et al. 2008). This means that granule cells transmit distinct outputs that depend on the specific combination of inputs they receive rather than acting as a simple relay, thereby enriching sensory representations for further cerebellar processing (Chabrol et al. 2015). The granule cell layer is thought to facilitate pattern separation through the connections of single mossy fibers to many granule cells, and their output is passed to PCs via parallel fibers (granule cell axons).

Granule cells have also been shown to encode non-sensorimotor, predictive information. They are able to encode the expectation of reward (Wagner et al. 2017), and also encode an acquired conditional response following eyeblink conditioning training (Giovannucci et al. 2017). This suggests that prediction is apparent even at the input stages of information processing within the cerebellar cortex.

Golgi cells, found in the granule cell layer, provide inhibition to granule cells and receive excitatory input from both mossy fibers and parallel fibers (Fig. 1). Golgi cells therefore provide both feedforward inhibition (as a result of their mossy fiber inputs) and feedback inhibition (via granule cell axon and parallel fiber inputs) to their target granule cells (D'Angelo 2008).

3.1.2 Parallel Fiber Inputs to the Molecular Layer

Granule cell axons bifurcate to form parallel fibers that extend along the molecular layer (Fig. 1, Sect. 2.2.2). Parallel fibers are slowly conducting but evoke rapid excitatory responses in all neurons in the molecular layer, including PCs, molecular layer interneurons (MLIs), and the dendrites of Golgi cells (Jirenhed et al. 2013). Owing to the large number of granule cells, each PC is estimated to be contacted by around 150,000 parallel fiber synapses (Zang and De Schutter 2019), although many of these may be functionally weak or silent (Isope and Barbour 2002). The parallel fiber–PC synapse is an important site of plasticity, which is discussed further in Sect. 3.4.1.

Parallel fiber inputs to MLIs provide feedforward inhibition onto PCs, which can modulate the efficacy of parallel fiber inputs (Binda et al. 2016). Maintenance of the excitatory–inhibitory balance of PC inputs is important for cerebellar functioning, given silencing MLIs' changed firing patterns of PCs, increasing simple spike rate and regularity, and impaired locomotor behavior (Jelitai et al. 2016). Basket cells and stellate cells are both subtypes of MLIs, and they may have different impacts on PC signaling. Removing GABAergic transmission from basket cells of behaving mice was shown to increase simple spike rate in PCs while decreasing complex spike rate; the same manipulation in stellate cells increased the regularity of simple spikes and increased complex spike rate (Brown et al. 2019). Therefore, MLI subtypes are likely to have different roles in cerebellar information processing.

3.1.3 Purkinje Cell Simple Spikes and Complex Spikes

Climbing fibers provide an incredibly powerful excitatory synaptic input to PCs and as a result generate unique action potentials known as complex spikes (Fig. 3; Eccles et al. 1966; Thach 1967). While the simple spikes generated by a PC resemble typical action potentials (duration one to two milliseconds), complex spikes generated by the same PCs are longer in duration (approximately 10 milliseconds on average) and consist of an initial large sodium-dependent component followed by a variable number of smaller, calcium-dependent components known as spikelets (Fig. 3; Eccles et al. 1966). Simple spikes are generated both intrinsically and as a result of mossy fiber–parallel fiber inputs, and occur at a wide range of firing frequencies, ranging from 19 to 95 Hz in vivo (mean 44 Hz; Armstrong and Rawson 1979) and 1 to 148 Hz (mean 38.8 ± 2.4 Hz) in vitro, even in the absence of synaptic inputs (Hausser and Clark 1997).

By contrast, complex spikes arise solely as the result of climbing fiber input. They occur at approximately 1 Hz in the awake animal, and their occurrence causes a subsequent pause in simple spike firing, the duration of which may relate to the number of spikelets in the complex spike (Fig. 3; Burroughs et al. 2017). The secondary components of a complex spike can reach frequencies of 500–600 Hz (Campbell and Hesslow 1986) and vary considerably in number, but typically each complex spike has three or four spikelets (Burroughs et al. 2017). MLIs, which form inhibitory connections with PCs (Fig. 1), have been shown in rat cerebellar slices to

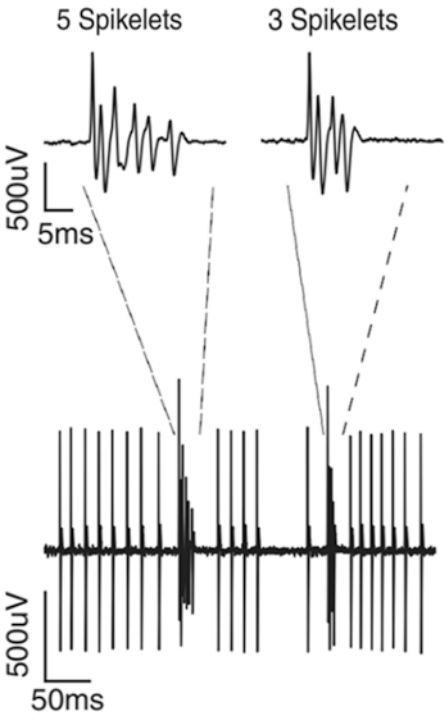

Fig. 3 An example extracellular recording from a single Purkinje cell showing complex spikes and simple spikes. The number of spikelets within a complex spike is variable, as shown in the insets. (Modified from Burroughs et al. 2017)

also receive inputs from climbing fibers as a result of glutamate spillover (Szapiro and Barbour 2007), so as well as providing direct excitatory inputs to PCs, climbing fiber inputs may also produce feedforward inhibition.

Inferior olive neurons, the origin of climbing fibers, are electrotonically coupled via gap junctions that synchronize their activity (Leznik and Llinás 2005). Synchronous activation of climbing fibers is thought to be important for the initiation and coordination of movement in mice (Hoogland et al. 2015), and greater complex spike synchrony increases the amplitude of complex spike-induced short-latency inhibitory and long-latency excitatory responses in CN neurons of anesthetized rats (Tang et al. 2019). Coupled PCs, as determined by correlations in complex spike occurrence, also have increased likelihood of simple spike synchrony (Wise et al. 2010). In addition, the number of spikelets in a complex spike correlates with synchronization (Lang et al. 2014), and the variability in spikelet number suggests that complex spikes are not an "all-or-nothing" event but instead convey information (Zang and De Schutter 2019). Thus, the complex spike activity of PCs, particularly when synchronized, can result either directly or indirectly in changes in cerebellar output, which in turn has the potential to influence behavior. The role of complex spikes and simple spikes in behavior is further discussed in Sect. 5.3.4.

3.1.4 Purkinje Cell Targets Within the Cerebellar Cortex

In mice, PCs have been found to directly inhibit other cell types in the cerebellar cortex via axon collaterals in the parasagittal plane, including neighboring PCs, MLIs, and Lugaro cells (Witter et al. 2016), thereby regulating their own inputs. In terms of cerebellar cortical non-uniformity, there may also be regional differences in how PCs regulate their own feedback. For example, PC axon collaterals directly inhibit granule cells in localized regions of the cerebellum related to eye movements and vestibular processing (lobule X, ventral paraflocculus, and flocculus; Guo et al. 2016). As well as these local synaptic connections, studies in mice have also shown that climbing fiber synapses to one PC can generate large negative extracellular signals that suppress simple spikes in neighboring PCs via ephaptic coupling (Han et al. 2020). This means that a single climbing fiber may in fact influence multiple local PCs, which may, in turn, promote firing of cerebellar output neurons due to synchronous disinhibition.

Connections between cells of the same type are a common feature in the cerebellum. As well as PCs targeting other PCs as described above, the same principle also holds true for cerebellar cortical interneurons. MLIs and Golgi cells are connected to the same cell type by both electrical (gap junction) and chemical (GABAergic) synapses (Mann-Metzer and Yarom 1999; Rieubland et al. 2014)—the former promoting synchronization in Golgi cells (Dugué et al. 2009). The extent to which this reciprocity is important for cerebellar function requires further investigation.

3.2 Purkinje Cell Control of Cerebellar Nuclei

PCs are the sole output of the cerebellar cortex, projecting to either the CN or vestibular nuclei, and are therefore central to cerebellar information processing. They are inhibitory, using GABA as a neurotransmitter, and form the main synaptic input to CN neurons with approximately 60% of synaptic boutons in the CN arising from PC axons (Ito 1984). Other synaptic inputs to CN are mainly from glutamatergic climbing fiber and mossy fiber collaterals (Fig. 1). In mice, excitatory climbing fiber collateral inputs to CN neurons resulting from olivary stimulation have been shown to be overridden by climbing fiber-induced inhibition via the PC pathway (Lu et al. 2016), suggesting that PC inhibition is the dominant CN input.

PC complex spikes in vivo have been shown to exert a strong and long-lasting inhibitory effect on activity in some CN neurons, although in others there is an excitatory–inhibitory sequence. Andersson and Oscarsson (1978), who first identified microzones in the B zone of the cat cerebellum, showed that lateral vestibular nuclear neurons were activated by collaterals of climbing fibers projecting to PCs providing inhibition to the same group of neurons. Therefore the same climbing fiber axons can produce excitation of CN neurons via direct collateral inputs followed by indirect inhibition via PC inputs (Blenkinsop and Lang 2011).

In young rat cerebellar slice preparations, hyperpolarizing currents (mimicking PC inhibition) are able to reduce spontaneous activity of CN neurons and subsequently elicit a rebound depolarization (Aizenman and Linden 1999). In mice this depolarization is thought to underlie rebound increases in CN firing rate that occur in vivo following trains of stimuli delivered to the cerebellar cortex or the inferior olive (Hoebeek et al. 2010). Similarly, in cat, synchronous climbing fiber activation evoked by electrically stimulating the peripheral receptive field results in substantial inhibition of CN neurons followed in some cases by rebound responses, suggesting this may be an important feature of the olivo-cortico-nuclear system (Bengtsson et al. 2011). However, it remains a matter of debate whether rebound firing occurs under physiological conditions because it has been less reliably observed in vivo as compared to in vitro investigations, and often involves non-physiological patterns of stimulation (Alvina et al. 2008; Witter et al. 2013).

Other studies argue that asynchronous PC inputs suppress CN firing while synchronous activity can entrain nuclear firing to PC inputs (Person and Raman 2012a, b), with single stimuli to the cerebellar cortex in rodents evoking precisely timed action potentials without changing firing rate (Hoebeek et al. 2010). The net effect of PC inhibition on individual CN neurons therefore likely depends on the degree of PC synchrony together with the level of PC–CN convergence (Tang et al. 2016). Further study is required to clarify how these factors contribute to cerebellar functions, and to determine if the mechanisms of PC to CN signaling are consistent throughout the cerebellum.

The nature of PC influence on the CN is also complicated by the presence of multiple cell types within the CN, including: (1) large glutamatergic neurons projecting to extracerebellar targets to provide powerful and short-latency excitatory

connectivity, notably with the thalamus and red nucleus, to influence motor and premotor areas; (2) small GABAergic projection neurons, which are the origin of the topographically organized nucleo-olivary inhibitory projection mentioned above (Uusisaari and Knopfel 2011); (3) glycinergic premotor output neurons in the medial nucleus (Bagnall et al. 2009); (4) inhibitory projection neurons of the interpositus nucleus, which have been recently described in mice, with inputs to regions including the pontine nuclei, medullary reticular nuclei, and sensory brainstem structures, such as the external cuneate nucleus, cuneate nucleus, parabrachial nuclei, and vestibular nuclei (Judd et al. 2021); (5) a heterogeneous population of local interneurons including an inhibitory population with a mixed GABAergic and glycinergic phenotype (Husson et al. 2014), and a non-GABAergic (putatively glutamatergic) population (Uusisaari and Knopfel 2012); and (6) nucleo-cortical neurons, which project to the cerebellar cortex and can be inhibitory or excitatory as outlined in Sect. 2.2.2 (Ankri et al. 2015; Houck and Person 2014).

There is evidence that the effects of PC inhibition on CN neurons may depend on cell type. In vivo recordings in mice suggest that glutamatergic cells of the medial nucleus respond to the rate and timing of PC inputs, with synchronous PC activation entraining CN neuron activity, whereas GABAergic neurons respond to mean population firing rates and may therefore encode PC inhibition differently (Özcan et al. 2020). A key outstanding question is how different cell types across the nuclei respond to their synaptic inputs, and how they interact with one another to shape CN output.

3.3 Zebrin Stripes

Several important physiological differences have been found in vivo in rodents between zebrin II positive (Z+) and negative (Z–) PCs (see Sect. 2.3). Firstly, simple spike firing rates are higher on average in Z– PCs than Z+ PCs (Zhou et al. 2014; Xiao et al. 2014). Secondly, the climbing fiber-evoked pause in simple spike firing is shorter in duration in Z– PCs (Xiao et al. 2014). And thirdly, the regularity of simple spike firing rates is greater in Z– PCs (Zhou et al. 2014; Xiao et al. 2014). These systematic differences in simple spike activity are thought to be due to the presence of the transient receptor potential cation channel C3 (TRPC3) in Z– PCs, since blocking these channels pharmacologically reduces simple spike firing rates in Z– but not Z+ PCs (Wu et al. 2019). Mice with loss of TRPC3 function show impaired eyeblink conditioning, a form of cerebellar learning that occurs in cerebellar cortical regions associated with Z– PCs, whereas compensatory eye movement adaptation, related to Z+ regions, remains intact (Wu et al. 2019). This suggests important differences in function of Z+ and Z– PCs, reinforcing the notion that cerebellar cortical physiology is not uniform.

Complex spike firing rates are also higher on average in Z– PCs than in Z+ PCs. Moreover, complex spikes have a longer half-width, implying a larger number of spikelets, and larger spike area in Z+ PCs (Zhou et al. 2014). Z+ PCs in vitro display

prolonged excitation following climbing fiber activation due to terminals in Z+ regions having larger pools of release-ready vesicles and enhanced multi-vesicular release, thus triggering longer-duration complex spikes with a greater number of spikelets (Paukert et al. 2010). However, the same phenomenon was not observed in vivo with Z+ and Z– PCs having similar distributions of spikelet number (Tang et al. 2017). Z+ PCs also show a greater variety of simple spike responses following a complex spike (Zhou et al. 2014), which may be related to differences in mossy fiber–granule cell–parallel fiber inputs, MLI inputs, and/or differences in PC intrinsic excitability.

The systematic differences in zebrin expression in the cerebellar cortex are also retained in some regions of the CN. The lateral, posterior interpositus and caudal medial nuclei contain terminals of Z+ PCs, whereas the anterior interpositus and rostral medial nucleus receive Z– PC terminations (Sugihara 2011). It might be expected that by comparison to Z+ PCs, Z– PCs cause more inhibition in their target CN neurons due to their higher firing rates and therefore Z– PC targets would have lower firing rates; however, recent research suggests the opposite. In awake adult mice, firing rates are consistently lower in CN neurons receiving input from Z+ PCs than those with input from Z– PCs (Beekhof et al. 2021). Identifying the reason(s) for this difference could enhance our understanding of how information is processed in cerebellar modules related to different behaviors.

In addition to Z+ and Z– PCs having distinct physiology, there is also evidence that zebrin stripes can act together in functional pairs; in the pigeon vestibulocerebellum, pairs of Z+ and Z– bands form functional units in relation to patterns of optic flow, e.g., self-rotation about the vertical axis (Graham and Wylie 2012). It remains to be determined exactly how zebrin II-related differences in physiology relate to differences in output, and ultimately function, throughout the cerebellum.

3.4 Synaptic Plasticity

3.4.1 Parallel Fiber–Purkinje Cell Synaptic Plasticity

Plasticity in the cerebellum was first studied at the parallel fiber–PC synapse, where long-term depression (LTD) was found to be induced by paired stimulation of parallel fibers and climbing fibers in vitro (Marr 1969; Albus 1971; Ito and Kano 1982). Ito et al. (1982) showed that LTD at this synapse could be induced in vivo by coincident stimulation of the sources of mossy fibers and climbing fibers, the vestibular nerve and inferior olive respectively, to the flocculus in decerebrate rabbits.

The mechanisms of LTD are described in detail in a review by Hoxha et al. (2016). In brief, parallel fiber–PC LTD requires postsynaptic calcium influx resulting from climbing fiber input together with intracellular release of calcium resulting from activation of mGluR1 by glutamate released from parallel fibers (which also activates AMPA receptors). The increase in intracellular calcium leads, via a biochemical cascade involving protein kinase C, in the endocytosis of postsynaptic AMPA recptors in PCs. This renders the PC less responsive to parallel fiber inputs.

Long-term potentiation (LTP) can also be induced at this synapse by stimulation of parallel fibers alone, typically at 1 Hz, which leads to insertion of AMPA receptors into the postsynaptic membrane (Salin et al. 1996; Lev-Ram et al. 2002). This type of stimulation results in relatively low levels of intracellular calcium compared to the LTD protocol described above, promoting activation of protein phosphatases and binding of N-ethylmaleimide-sensitive factor to AMPA receptors, leading to the stabilization of these receptors in the postsynaptic membrane (Hoxha et al. 2016). In fact, both LTD and LTP can occur at most synapses within the cerebellum through a variety of mechanisms (for reviews, see Mapelli et al. 2015; Gao et al. 2012).

Parallel fiber–PC synaptic transmission is dysfunctional in rodent models of spinocerebellar ataxia, for example because of abnormal regulation of mGluR1 (SCA1, SCA5) or deficient release of glutamate from parallel fibers (SCA27, Hoxha et al. 2016). Physiological functioning of this synapse is therefore central to normal cerebellar function and genetic causes of ataxia may lead to its dysregulation. The roles of parallel fiber–PC plasticity in behavior are further explored in Sect. 5.3.1.

3.4.2 Zebrin II and Synaptic Plasticity

LTD is thought to be the predominant form of parallel fiber–PC plasticity in regions of cortex containing Z– PCs because of their relatively high baseline firing rate, while for Z+ PCs their lower firing rate predisposes them to LTP (De Zeeuw and Ten Brinke 2015; De Zeeuw 2021). Zebrin II colocalizes with EAAT4, a transporter that limits the duration of action of glutamate at the synapse, and this reduction of glutamate prevents LTD by reducing activation of metabotropic glutamate receptors. Consistent with this transmitter regulation, studies in rat cerebellar slices, in which parallel fiber stimulation was paired with PC depolarization, induced LTD of parallel fiber inputs to Z– PCs (in lobule III), but under the same conditions did not elicit plasticity at parallel fiber inputs to Z+ PCs (in lobule X; Wadiche and Jahr 2005). EAAT4 is also likely to influence other cerebellar cortical signaling, as it regulates glutamate spillover from climbing fibers to MLIs (Malhotra et al. 2021). Such findings therefore strongly suggest that the same synaptic transmission and plasticity rules are not likely to apply to the cerebellum as a whole.

3.4.3 Plasticity at Cerebellar Nuclei Synapses

Studies in mice in vitro have shown that LTP can be induced at the mossy fiber–CN synapse by high-frequency stimulation of mossy fibers combined with hyperpolarization of the postsynaptic CN neuron, which in turn leads to rebound firing (Person and Raman 2010; Pugh and Raman 2006). By contrast, LTD can be induced at the same synapse via high-frequency stimulation either with or without depolarization of the postsynaptic CN neuron (Zhang and Linden 2006). LTP (Ouardouz and Sastry 2000) and LTD (Morishita and Sastry 1996) can also be induced at PC inputs to CN neurons. Indeed, one study has shown that a particular burst protocol can

induce both LTP and LTD at PC synapses onto CN neurons in rat cerebellar slices, depending upon the level of postsynaptic excitation (Aizenman et al. 1998). The direction of plasticity (LTP versus LTD) depends on the state of the postsynaptic CN neuron, which suggests that the level of inhibition from PCs (and potentially local interneurons) regulates plasticity of excitatory synapses to CN neurons. Such an arrangement could be a homeostatic mechanism to maintain synaptic strength within an operational range.

As both LTP and LTD can occur at various nodes within the cerebellar circuitry, this creates a high level of complexity that goes beyond the classical view of LTD at the parallel fiber–PC synapse being the key mechanism of cerebellar synaptic plasticity (for more detail, see Sect. 5.3.1). The balance of increases and decreases in plasticity, which lead to changes in cerebellar output, depends upon the timing of synaptic inputs, the state of excitability of the neurons, and the cerebellar region of interest. Little is known about how different forms of plasticity interact with one another to influence cerebellar information processing.

4 Systems Physiology

4.1 Somatotopic Organization

Central to cerebellar function, particularly for its contributions to motor control, is how the cerebellum receives sensory inputs from the body and sense organs. The first systematic report of a somatotopic organization in the cerebellum was by Snider and Stowell (1944) who recorded, in the anesthetized cat and monkey, field potentials on the cerebellar surface evoked by peripheral tactile stimulation, and observed responses in discrete regions of the cerebellar cortex that were organized in a similar pattern in both species—one map in the anterior lobe and two more in the posterior lobe of the cerebellum. A similar somatotopic organization has subsequently been described in a range of other species, notably the rat (Atkins and Apps 1997; Jörntell et al. 2000), and fine-resolution mapping has shown that this somatotopy corresponds to cerebellar cortical zones (Sect. 2.2.1). More recently, non-invasive functional magnetic resonance imaging (fMRI) has shown, albeit at a coarser level of spatial resolution, that the same general somatotopic arrangement is also present in the human cerebellum (Fig. 4, Grodd et al. 2001; Ashida et al. 2019; Boillat et al. 2020).

A basic somatotopy also exists within the CN, and therefore in the cerebellar output. For example, in cats and monkeys the fore- and hindlimbs are represented in posterior and anterior regions, respectively, of the anterior interpositus (van Kan et al. 1993; Garwicz and Ekerot 1994), while in dentate both face and eyes are represented (van Kan et al. 1993). However, in other CN regions a somatotopy is not evident. For example, in the posterior interpositus in monkeys there is no clear separation between representation of the fore- and hindlimbs (van Kan et al. 1993).

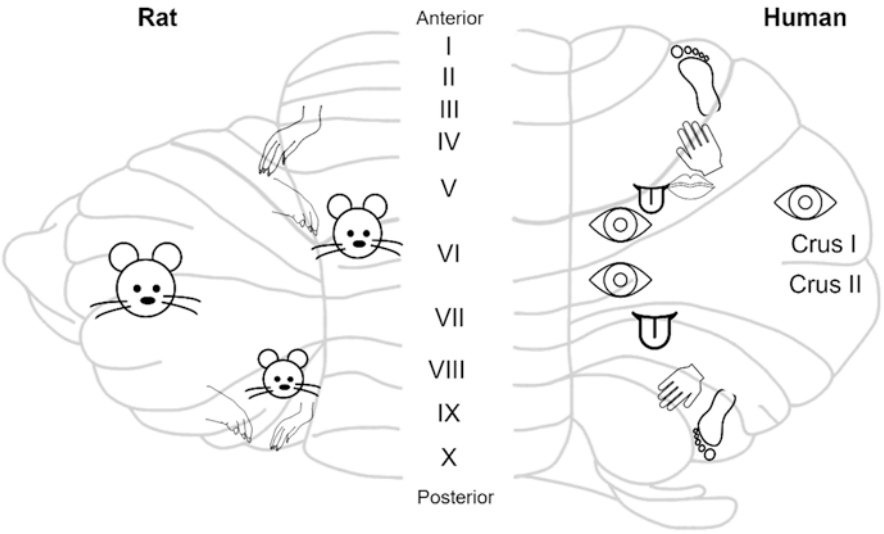

Fig. 4 Somatotopic organization in a dorsal view of the rat and human cerebellar cortex. Left shows approximate locations of representations of the hindlimb, forelimb, and face in the rat based on works by Atkins and Apps et al. (1997), Jörntell et al. (2000), and Bosman et al. (2010), determined by electrophysiological responses to peripheral stimulation. Right shows approximate representations of the foot/toes, hand/digits, tongue, eyes, and lips as described by Boillat et al. (2020) and Grodd et al. (2001), determined using fMRI in participants voluntarily moving specified body parts

The somatotopy within the CN has been studied in most detail in the anterior interpositus in the cat and a finer map of the ipsilateral forelimb is present that relates to the olivo-cortico-nuclear projections of the paravermal C1, C3, and Y modules (Garwicz and Ekerot 1994). Ekerot et al. (1995) found that microstimulation of these different CN regions in cat elicited different patterns of multi-segmental ipsilateral forelimb movement. This suggests that, at least for paravermal regions, the cerebellar control of movements is organized in a modular framework. Consistent with this possibility, recent research in mice has shown that a small area in the rostral part of the anterior interpositus controls motor synergies that protect the eye during eyeblink conditioning by coordinating the eyelid, neck, and forelimb muscles, and that individual CN neurons encode information related to all these effectors (Heiney et al. 2021). This provides support to the general concept that cerebellar maps are related more to actions of different body parts rather than being strictly somatosensory (Apps and Garwicz 2005).

As outlined above (Sect. 2.2.2), anatomical studies have provided evidence that mossy fibers generally align with the climbing fiber organization in the cerebellum. However, detailed electrophysiological mapping of multiunit granule cell activity driven by mossy fiber inputs in anesthetized rats has also revealed a “fractured somatotopy” of tactile responses whereby different body parts are represented in a patchy mosaic pattern in the hemispheres of the cerebellar cortex (Shambes et al. 1978; Kassel et al. 1984; Apps and Hawkes 2009). The apparent discrepancy

between a fractured and a more systematic somatotopic arrangement could be the result of several possibilities including regional differences in cerebellar physiology (paravermal versus hemispheral cortex), and the extent to which local granule cells have their main influence on overlying PCs or on PCs in other regions of cortex via their parallel fibers. Further studies are required to investigate these and other possibilities.

In summary, the somatotopic organization of inputs to and outputs from the cerebellum suggests that the physiological organization of cerebellar connectivity is highly conserved across species, and that output of different cerebellar regions is likely to subserve control of coordinated movements that may involve a combination of different body parts. However, the way CN interact with the rest of the central nervous system to coordinate complex movements remains poorly understood.

4.2 Physiologically Defined Olivocerebellar Pathways

Electrophysiological studies have revealed a complex array of spino-olivocerebellar pathways (SOCPs, Fig. 5) that transmit information from skin, muscle, and joints to the inferior olive, which in turn forward this information to the cerebellum via climbing fibers (Oscarsson 1980; Ito 1984). Transmission in SOCPs can be recorded as evoked climbing fiber field potentials in the cerebellum, and combined electrophysiological mapping and anatomical tract tracing experiments have shown that cerebellar cortical zones defined by their SOCP input and those defined anatomically by their olivo-cortico-nuclear connectivity are largely congruent (e.g., Trott and Armstrong 1987a, b; Trott and Apps 1991, 1993; Edge et al. 2003).

A similar arrangement occurs for descending inputs from the cerebral cortex, which collectively are termed cerebro-olivocerebellar pathways (COCPs, Fig. 5). COCPs originate in many regions of the cerebral cortex and project to the inferior olive via the mesodiencephalic junction (Wang et al. 2022). COCPs also conform to the zonal organization of the cerebellum; they converge on the same olivary regions that supply climbing fibers to the cortical zones defined by the SOCPs (Andersson and Nyquist 1983). For example, stimulation of the ipsilateral forelimb and the somatotopically corresponding region of the contralateral motor cortex results in convergent cerebellar climbing fiber responses in the forelimb-receiving part of the C1 zone in the rat (Ackerley et al. 2006).

The source of climbing fibers, the inferior olive, receives a variety of sensory and motor inputs from regions including the trigeminal nuclei, dorsal column nuclei, red nuclei, cerebral cortex (via the mesodiencaphalic junction, as described above), CN, and spinal cord (De Gruijl et al. 2013). This convergence of inputs, together with the fact that COCPs conform to the zonal organization of the olivocerebellar system, reinforces the close relationship between ascending and descending pathways to the cerebellum and highlights the potential role of the inferior olive as a comparator of these two sources of information (Oscarsson 1980; for further discussion, see Sect. 6).

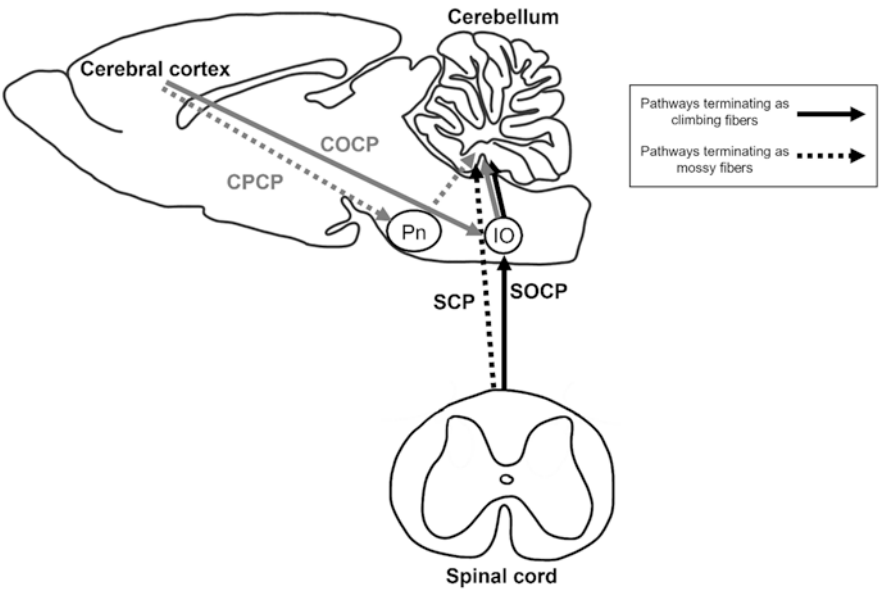

Fig. 5 Simplified diagram of cerebellar input pathways as depicted on a rat brain and spinal cord. Spino-olivocerebellar pathways (SOCPs) carry information from the spinal cord to the inferior olive (IO), which send climbing fiber inputs to the cerebellum. These ascending pathways include both direct spino-olivary projections and indirect pathways via various brainstem relays including the dorsal column nuclei. Cerebro-olivocerebellar pathways (COCPs) also provide climbing fiber input to the cerebellum via the inferior olive but originate in the cerebral cortex and include descending pathways that relay in a range of brainstem nuclei including the midbrain tectum. Mossy fibers include both direct and indirect projections from the spinal cord (spinocerebellar pathway, SCP), and indirect projections from the cerebral cortex via the pontine nuclei (Pn) in the cerebro-pontocerebellar pathway (CPCP)

The flow of information via SOCPs and COCPs is modulated during active movements. In particular, a series of studies in awake behaving cats has shown that separate modulatory drives act on the climbing fiber pathways that target the paravermal zones (Apps et al. 1990, 1995, 1997; Lidierth and Apps 1990; Apps and Lee 1999; Pardoe et al. 2004). For example, low-intensity electrical stimulation of the ipsilateral superficial radial nerve in cats evokes SOCP-mediated field potentials in the cerebellar cortex, which vary systematically in size throughout the step cycle. By comparison to rest, responses recorded in the C2 zone were usually smallest (implying reduced SOCP transmission) in the swing phase of the step cycle in the ipsilateral forelimb (Apps et al. 1990). In contrast, in the neighboring C1 zone, the smallest responses consistently occurred during the stance phase in the ipsilateral forelimb (Lidierth and Apps 1990). Such differences suggest functional variations between zones, and it is thought that the gating of sensory inputs serves to prevent the transmission of self-generated, predictable signals in a task-dependent manner (Lawrenson et al. 2016).

4.3 Spinocerebellar Mossy Fibers

Cerebellar mossy fibers arise via multiple pathways including spinocerebellar, cuneocerebellar, reticulocerebellar, and cortico-pontocerebellar tracts (Fig. 5). Spinocerebellar pathways provide proprioceptive information from skin, muscle, and joints to the cerebellum (Bloedel and Burton 1970; Snyder et al. 1978), but may encode information about movement of a body region, e.g., a whole limb rather than individual joints or muscles (Bosco and Poppele 2001). The importance of these pathways is emphasized by the fact that they include some of the fastest conducting axons in the central nervous system, with degeneration of spinocerebellar tracts resulting in profound disorders to movement control, characterized by Friedreich's ataxia.

4.4 Cerebro-Cerebellar Pathways

The cerebellum receives substantial inputs from the cerebral cortex mainly via pontine nuclei (cerebro-pontocerebellar pathways; Fig. 5) and the inferior olive (COCPs). The cerebellum also sends projections to the cerebral cortex, predominantly via the thalamus, and these reciprocal cerebro-cerebellar connections likely contribute to the variety of cerebellar functions that extend beyond the motor domain (Strick et al. 2009). Recent evidence for reciprocal cerebro-cerebellar interactions has found, for example, that neurons in the CN display preparatory ramping activity related to planning future movement when holding a short-term memory, or anticipating a reward, as is known to occur in the frontal cortex (Gao et al. 2018; Chabrol et al. 2019). Inactivating the cerebellar fastigial nuclei (Gao et al. 2018) or lateral nuclei (Chabrol et al. 2019) disrupts preparatory activity in the frontal cortex, and inactivating the frontal cortex abolishes preparatory activity in the CN. Such findings therefore point to cerebro-cerebellar communication being important for anticipating and planning future actions.

An increasing number of studies have studied neural oscillations in cerebro-cerebellar circuits. Oscillations are rhythmic patterns of synchronous neural activity that can occur within local circuits and also between distant brain regions. They are thought to be important for input selection, plasticity, and communication between brain regions (Buzsáki and Draguhn 2004; Fries 2015). In the cerebellum oscillations occur at a wide range of frequencies (De Zeeuw et al. 2008), although their functional significance remains far from being clear. However, during whisking in rats, inactivation of the rat cerebellum disrupts coherent neural oscillations between the sensory and motor cortices (Popa et al. 2013). This raises the interesting possibility that the cerebellum may modulate cerebral processing by coordinating communication between cerebral regions. Clearly, however, much remains to be done to gain a full understanding of the functional significance of oscillatory activity within cerebro-cerebellar circuits.

5 Behavioral Physiology

5.1 Limb Control

5.1.1 Locomotion

The cerebellum is involved in the control of locomotion, and accordingly cerebellar damage can result in ataxia (Morton and Bastian 2004). Studies of cerebellar neuronal activity during locomotion, mainly in cats during treadmill or horizontal ladder walking, have found that PCs discharge simple spikes rhythmically in a manner that is time locked to the step cycle. Typically, PCs have one period of increased simple spike discharge per step, but some may have two or three peaks (Armstrong and Edgley 1984b), and the timing of discharge differs between cerebellar cortical regions.

In the vermal B zone in decerebrate cats, Udo et al. (1981) reported two profiles of PC simple spike discharge—one population of PCs had a peak of activity in the late swing or early stance phase of the step cycle of the ipsilateral forelimb, and a second population had two peaks: one during late swing, the other during late stance/early swing. The pattern of activity during late swing may relate to the preparation of limb touchdown, consistent with vermal regions of the cerebellum being involved in maintenance of stance and balance (Morton and Bastian 2004). Moreover, while the pattern of complex spike activity in B zone PCs in awake cats occurs without any clear relationship to the step cycle, an increase in probability occurs following a perturbation, as has been shown following an unexpected rung drop during horizontal ladder walking (Andersson and Armstrong 1987). This suggests that climbing fibers can signal unexpected events or "errors" during a predictable movement (see Sect. 5.3.1).

In the neighboring C1 zone in the paravermis, the peak of simple spike activity consistently occurs during the swing phase of the ipsilateral forelimb (Armstrong and Edgley 1984b). Activity of neurons in the anterior interpositus, which receive projections from C1 zone PCs, follows the same pattern of activity (Armstrong and Edgley 1984a, b). This suggests that, instead of shaping CN activity through inhibition, PCs may instead dampen excitatory drive to CN neurons in this case (for other ways in which PCs may influence CN activity, see Sect. 3.2). Simple spike activity of PCs in the paravermal C2 and C3 zones occurs slightly later in the step cycle, with peak activity at the transition between the stance and swing phases (Edgley and Lidierth 1988). Differences are also present across the mediolateral width of the C2 zone (Edgley and Lidierth 1988). This suggests that, rather than a systematic shift in the patterns of activity between cortical zones, there is a mediolateral gradient, with more medially located PCs in the paravermis discharging earlier in the step cycle.

In keeping with this trend, PCs in the hemispheral D zones tend to have peak activity in the swing phase of the step cycle of the ipsilateral forelimb while walking on a horizontal circular ladder (Marple-Horvat and Criado 1999). The same is also

the case for dentate nuclear cells. Thus, in agreement with Armstrong and Edgley (1984b), the pattern of modulation of nuclear activity parallels that of the overlying PCs from which the cells receive inhibitory input (Marple-Horvat and Criado 1999). Approximately 45% of lateral cerebellar neurons (cortical and nuclear) were found to be responsive to visual cues, over two-thirds of which also showed rhythmic modulation in relation to the step cycle (Marple-Horvat et al. 1998). Taken together this suggests that D zone activity may be related to visually guided coordination of eye and body movements (Marple-Horvat and Criado 1999), consistent with control of whole body movements discussed above (Sect. 4.1).

Studies in rats freely traversing a track confirm that PC activity in lobules V and VI of the vermis is rhythmic during locomotion, and in addition that the patterns of modulation become variable due to other behavioral factors such as speed and acceleration (Sauerbrei Britton et al. 2015). Rhythmic patterns of cerebellar activity are therefore present during locomotion with a forced rhythm or stepping distance, as is the case with treadmill and ladder walking described above, and that which is self-initiated.

5.1.2 Reaching

Cerebellar disorders can result in deficits in reaching and grasping movements (Nowak et al. 2013; Zackowski et al. 2002). Cerebellar neurons, particularly those in paravermal regions, encode various components of single and multi-joint limb movements involved in reaching but the patterns of activity are quite mixed. For example, in monkeys performing arm and hand-based targeting tasks, activity of PCs is often modulated in relation to movement velocity, but may also correlate with position or acceleration, and can be tuned to preferred direction(s) (Hewitt et al. 2011; Marple-Horvat and Stein 1987; Fortier et al. 1989). The change in activity usually precedes movements, and an increase in PC discharge is most common with bursts of simple spikes positively correlated with limb muscle activity, although a proportion of PCs also show decreases in activity (Holdefer and Miller 2009). Generally speaking, individual PCs therefore display quite variable patterns of activity during even stereotypical movements such as reach-to-grasp. The reason for this variability is unclear but may in part be due to sampling PCs from different cerebellar zones (as is the case for studies of locomotion). Further investigations are needed in which PCs are studied in relation to cerebellar cortical modules and their microzonal subunits in order to gain a full understanding of cerebellar information processing.

Activity of CN neurons can also precede reaching movements and correlate with velocity, position, and acceleration, as well as have a preferred direction (Marple-Horvat and Stein 1987). A large proportion of interpositus neurons in the monkey increase their activity preferentially during a reach-to-grasp movement but only when the movement included grasping (van Kan et al. 1994). This suggests that the interpositus may be important in the control of grasping an object but not necessarily in the control of directing the limb to grasp the object. In contrast, a more recent

study in mice performing a forelimb reaching task found that the activity of interpositus neurons was modulated near the endpoint of a reach and was therefore likely related to limb deceleration to enhance accuracy (Becker and Person 2019). Besides possible species differences, subpopulations of neurons within the cerebellar nuclei (potentially relating to the output of different cerebellar modules) may encode different aspects of complex, multi-joint limb movements, including activation or inactivation of relevant effector muscles.

Other cerebellar regions may also encode reaching-relevant information. For example, PCs in the D2 zone of the lateral cat cerebellum encode visual information related to a predictable moving target, and this activity continues even in the temporary absence of the target, consistent with PCs encoding a feedforward prediction (internal model) of the expected movement (Cerminara et al. 2009). The role of the cerebellum as a feedforward controller is further discussed in Sect. 6.

5.2 Eye Movements

The posteromedial cerebellum, flocculus, and paraflocculus are involved in the optimization of eye movements, including saccades and smooth pursuit, via vestibular nuclear inputs to oculomotor neurons. As such, saccade dysmetria and nystagmus are often observed in cerebellar patients (Moscovich et al. 2015). Caudal fastigial nucleus neurons in the monkey discharge a burst of action potentials for almost every saccade, and the timing of these bursts suggests this signal is related to the start of contraversive saccades and the end of ipsiversive saccades—perhaps relating to acceleration and deceleration respectively (Fuchs et al. 1993; Robinson and Fuchs 2001). Caudal fastigial activity precedes smooth pursuit onset, and floccular PCs burst after the onset of movement so may be involved in maintaining smooth pursuit (Robinson and Fuchs 2001). In accordance with its control of eye movements, one classical example of cerebellar learning is the vestibulo-ocular reflex (VOR), which is further described in Sect. 5.3.2.

5.3 Associative Learning

Early theories of cerebellar learning by Marr (1969) proposed that climbing fibers provide an "error" signal to the cerebellum to induce learning or refinement of movements, and were extended by Albus (1971) to suggest that this involved depression of parallel fiber–PC synapses. Ito and Kano (1982) showed that parallel fiber activation in conjunction with climbing fiber activation was able to induce LTD at this synapse in the cerebellum, using electrical stimulation in decerebrate rabbits, providing early evidence of cerebellar synaptic plasticity and the role of climbing fibers in cerebellar learning. It is now known that the interval between mossy fiber and climbing fiber signals, which induces plasticity, varies across cerebellar regions

(ranging from ~0 to 150 milliseconds), accounting for the range of feedback delays relevant to the behavioral functions of each region (Suvrathan et al. 2016).

Associative learning in the cerebellum can be demonstrated by Pavlovian classical conditioning, where a previously neutral conditioned stimulus (CS) elicits a behavioral response after repeated presentations with an unconditioned stimulus (US). The US is thought to be signaled to the cerebellum via the inferior olive by climbing fibers, while the CS is conveyed by mossy fibers via the pontine nuclei (Steinmetz et al. 1989). These two pathways converge in the cerebellum, in both the cerebellar cortex and CN, and the sites of anatomical convergence are thought to underlie learning of the association between the two inputs. In line with the roles of the cerebellum in eye movements, two of the most widely studied forms of learning involve the eye—eyeblink conditioning and the VOR.

5.3.1 Eyeblink Conditioning

Eyeblink conditioning is perhaps the best studied form of cerebellar learning. An air puff to the eye (US) is repeatedly paired with a CS, such as a tone, so that after learning the CS alone induces an eyeblink response. The underlying circuitry has been shown in a range of species to depend upon discrete cerebellar microzones in hemispheric lobule VI with cortico-nuclear projections to anterior interpositus. This cerebellar nuclear region, in turn, has projections to the facial nucleus via the red nucleus (Yeo et al. 1984, 1985a, b; Ten Brinke et al. 2019).

Studies of eyeblink conditioning have provided evidence of a clear link between changes in patterns of neural activity and learnt behavior. Presentation of a novel tone or light stimulus (equivalent to a CS) alone may evoke climbing fiber activity; however, this response reduces with repeated presentations as saliency decreases (Ohmae and Medina 2015). During CS–US pairings in early acquisition of eyeblink conditioning, however, the US (air puff) evokes a climbing fiber response that drives learning of the conditioned eyelid response in response to the CS; in later stages of learning, the eyelid closure is initiated after the CS in anticipation of the air puff (Sears and Steinmetz 1991; Medina et al. 2002). Once the conditioned response is acquired, climbing fibers fire in response to the predictive stimulus (CS) (Ohmae and Medina 2015). Conditioned eyelid closure is associated with simple spike suppression in PCs, which results in disinhibition of CN neurons (Johansson et al. 2014; Hesslow and Ivarsson 1994; Rasmussen et al. 2008). Increased activity of CN neurons during expression of the conditioned response subsequently inhibits climbing fiber activity driven by the US in the inferior olive, due to increased inhibition via nucleo-olivary projections (Medina et al. 2002; Rasmussen et al. 2008). During extinction learning, the CS is repeatedly presented without the US so it is learned that the expression of the defensive response is no longer required (Jirenhed et al. 2007). Climbing fiber inhibition resulting from the conditioned response (increased CN inhibition of the inferior olive) in the absence of the US is thought to be an important teaching signal for extinction (Ohmae and Medina 2015).

Long-term synaptic plasticity at parallel fiber to PC synapses is thought to underlie eyeblink conditioning, resulting in the suppression of PC firing as described

above. Paired stimulation of parallel fibers and climbing fibers, corresponding to the CS and US respectively, has been shown to induce LTD at parallel fiber–PC synapses, and interfering with metabotropic glutamate receptors and protein kinase C pathways involved in parallel fiber–PC LTD has been shown to inhibit synaptic plasticity and learning of the conditioned eyeblink response (Aiba et al. 1994; Koekkoek et al. 2003). However, Schonewille et al. (2011) found no significant impairment in eyeblink conditioning when a later step in the LTD pathway, AMPA receptor internalization, was blocked in three types of mutant mice. The authors argued that impairments produced by manipulating earlier steps in the LTD pathway may be related to cellular processes other than LTD. A subsequent study by Yamaguchi et al. (2016) using two of these mutant mouse models found that while conventional protocols did not induce LTD in these mice, LTD could be induced in vitro using intensified stimulation protocols, for example pairs of parallel fiber stimulations combined with PC depolarization at 1 Hz for 3 minutes (as opposed to a single parallel fiber and climbing fiber stimulus at 1 Hz for 5 minutes). Compensatory mechanisms may therefore be at play in mice with disrupted LTD mechanisms, although further experiments are required to investigate how any of these protocols relate to physiological LTD in vivo. In summary, despite the attractiveness of LTD at the parallel fiber–PC synapse being the cellular mechanism underpinning cerebellar contributions to motor learning, evidence remains lacking to show conclusively that this is the case.

5.3.2 Vestibulo-Ocular Reflex

The VOR is a gaze stabilizing reflex that produces eye movements opposing the direction of head movements. In an experimental setting, VOR can be manipulated by moving the head and visual inputs in the same or opposite directions at varying speeds and amplitudes, with eye movements adapting to each novel configuration to re-stabilize vision. The flocculus and ventral paraflocculus of the cerebellum receive visual error signals, which converge with vestibular and eye movement information (Frens et al. 2001; Noda 1986). Simple spike modulation of PCs in these regions correlates with head and eye position, and VOR adaptation drives changes in modulation of PC activity (De Zeeuw et al. 1995; De Zeeuw and Ten Brinke 2015). These PCs inhibit the vestibular nuclei, which project to oculomotor nuclei to control muscles of the eyes.

Genetically modified mice lacking protein phosphatase 2B, which is involved in parallel fiber–PC LTP and modifying the intrinsic excitability of PCs, show VOR adaptation deficits (Schonewille et al. 2010). Mice deficient in parallel fiber–PC LTD are still able to show VOR adaptations (Schonewille et al. 2011), suggesting that under such experimental conditions LTP may be the most important form of plasticity for this type of learning. However, this may not be true for all forms of VOR adaptation (for example, opposite gain modulation), with multiple forms of plasticity likely to contribute under physiological conditions when all signaling pathways are intact (Boyden et al. 2004; Kimpo et al. 2014).

5.3.3 Higher-Order Learning

Associative learning principles may also apply to higher-order forms of learning in which the cerebellum is involved—in particular, reward-based learning, as the cerebellum has been shown to encode several aspects of reward. For example, granule cells in mice encode expectation of reward (Wagner et al. 2017) and climbing fibers signal reward prediction in the lateral cerebellum (lobule simplex, Crus I and II) during learning (Heffley and Hull 2019). Climbing fibers can also signal reward delivery and omission, which map onto different cerebellar cortical microzones—reward delivery causes activation in a subset of microzones within lobules V and VI and suppression in others, whereas reward omission activates both sets of microzones (Kostadinov et al. 2020).

The reward omission signal conveyed by the climbing fiber system may be an "error" signal that occurs when the outcome is unexpected, in accordance with error-based theories of climbing fiber function (e.g., Zang and De Schutter 2019) and classical theories of cerebellar-dependent motor learning (Marr 1969). Supporting this idea, climbing fiber responses to predictable rewards are suppressed during learning (Kostadinov et al. 2020), and the phenomenon may be generalized to the cerebellar mossy fiber–granule cell–parallel fiber system because reward-related error signals in PC simple spike responses diminish as monkeys learn a reward-association task (Sendhilnathan et al. 2020).

During trial-and-error-based visuomotor association learning, PC simple spikes encode the outcome of the monkey's most recent decision throughout the subsequent trial, updating with each trial and decreasing with improved performance (Sendhilnathan et al. 2020). Cognitive learning in the cerebellum could therefore be driven by similar mechanisms as error-based motor learning, with learning in both motor and cognitive domains involving testing predictions against actual outcomes. However, this may not be true for all types of behavior given that climbing fibers do not always signal error, as explored in the next section.

5.3.4 Climbing Fibers and Learning

Providing an error signal may not be the universal function of climbing fiber inputs to the cerebellum, since varying patterns of PC complex spike activity during learning have been observed. For example, complex spikes in the posterior vermis of the monkey occur randomly before saccadic adaptation, yet a distinct response profile emerges (with an increase or decrease in probability of occurrence depending on direction of adaptation) during the adaptation process that may act to stabilize the learned behavior (Catz et al. 2005); the opposite might be expected if the complex spikes signaled error, as error signals would decrease with learning. In reward-driven behaviors, which require a cognitive component, complex spikes have been shown to signal reward prediction and thus may guide cerebellar learning in a feedforward, predictive manner (Heffley and Hull 2019).

Complex spike responses may adapt to respond preferentially to salient sensory cues over less behaviorally relevant cues (Bina et al. 2021). For example, complex spikes can adapt to occur in response to a tactile reward-related cue rather than a neutral auditory cue, which in turn promotes potentiation of simple spike responses to the salient cue (Bina et al. 2021). Climbing fibers, and resulting complex spikes, therefore can drive learning, but this may occur in different ways depending upon the type of behavior, the region of the cerebellum, and the larger brain networks involved. Simple spikes themselves may also signal error in motor behaviors as well as movement kinematics (Popa et al. 2012), providing an alternative route through which error-based learning may occur.

As outlined above (Sect. 3.1), climbing fibers have long been thought to carry an "all or nothing" signal (Eccles et al. 1966), but more recent work suggests that information may also be conveyed in the waveform of individual complex spikes (Burroughs et al. 2017; Zang and De Schutter 2019). For example, the amplitude of PC calcium signals triggered by climbing fiber synapses in mice is enhanced when there is a sensory event, which may drive plasticity (Najafi et al. 2014). Also, during learning of smooth eye pursuit movements in the monkey, longer climbing fiber bursts lead to longer-duration complex spikes (and by inference a larger number of spikelets) that promote plasticity and enhance learning (Yang and Lisberger 2014). Similarly, the number of spikelets in a complex spike (Fig. 3) has been shown to increase following acquisition of delay eyeblink conditioning in mice (Titley et al. 2020). Thus, the relationship between complex spikes and learning extends beyond an all or none action potential type event and is likely to contribute to the diversity of climbing fiber function in learning. Clearly this is a subject that merits further study, particularly in light of the systematic differences in complex spike waveform related to synchrony (Sect. 3.1) and also to zebrin topography (Sect. 3.4).

6 The Cerebellum as a Feedforward Controller

In terms of motor control, the cerebellum is thought to generate internal models (feedforward predictions) about the sensory outcomes of intended movements and update these using movement-related sensory feedback (Fig. 6). The sensory prediction error generated can then guide movements online, account for sensory reafference occurring from self-generated movement, and guide motor learning (Popa and Ebner 2019). The fact that loops such as cortico-nucleo-olivary circuits and reciprocal connectivity with regions of the cerebral cortex are at the heart of cerebellar connectivity makes it well suited as a feedforward controller. Recently described inhibitory projections from the interpositus nucleus to sensory-related areas in the brainstem (Sect. 3.2) add extra feedback loops that could in theory modulate predictions of actions and sensory reafference (Judd et al. 2021).

There is an increasing body of evidence to support the cerebellar feedforward prediction model, whereby a forecast of the consequences of an action is made before the action is completed (e.g., Miall and Wolpert 1996; Miall et al. 1998;

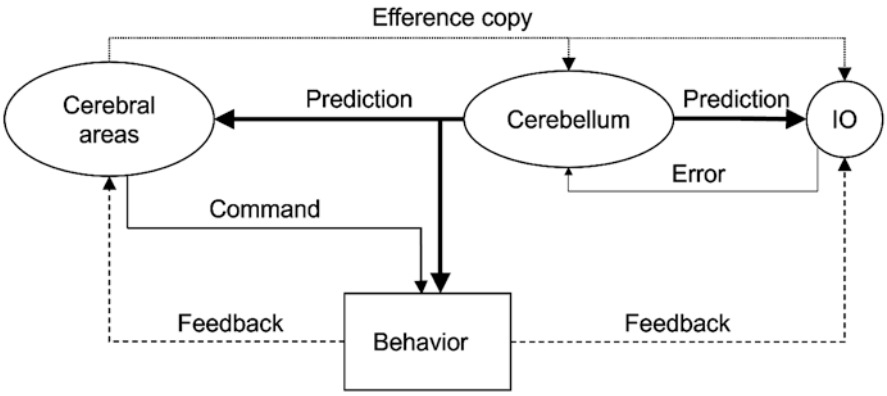

Fig. 6 A simplified schematic of circuits showing how the cerebellum acts as a prediction machine. Motor and non-motor actions such as goal-directed movements and problem solving occur following commands from cerebral areas (e.g., motor and prefrontal cortices, respectively) via connections to effector systems, such as the spinal cord for motor commands and association areas of the cerebral cortex for cognitive commands (behavior). An efference copy of these commands is sent to the cerebellum, via the pontine nuclei, and to the inferior olive (IO). Following behavior, feedback is delivered to the cerebellum via the IO and also to cerebral areas, and the IO compares cerebellar predictions to feedback to generate an error signal. The cerebellum compares the intended action (efference copy) and IO error signal to update its prediction. The prediction generated by the cerebellum allows online corrections of behavior through cerebellar connections to effector systems and updates the command in cerebral areas via the thalamus

Kitazawa et al. 1998; Cerminara et al. 2009; Ishikawa et al. 2016). In a recent example, recordings from a monkey during step tracking movements of the wrist found that activity in the dentate nucleus could predict the firing rate of mossy fibers, suggesting that the cerebellum is able to predict upcoming sensory inputs (Tanaka et al. 2019). There is also evidence from human subjects with cerebellar degeneration, who show impaired adaptive abilities during reaching and speech production compared to controls, suggesting problems with feedforward processing but not with compensatory responses, indicating that feedback systems are still intact (Parrell et al. 2021).

An extension of the internal model theory is to consider the cerebellum more generally as a "prediction machine" (Ramnani et al. 2000; Hull 2020). This expands cerebellar involvement in relatively simple circuits underpinning specific forms of motor learning, for example eyeblink conditioning, to more complex neocortical prediction paradigms involving interactions between multiple brain regions (Fig. 6). The reciprocal connections of the cerebellum with a multitude of brain structures provide the anatomical substrate to be involved in sensory, motor, and cognitive processes (Welniarz et al. 2021). It is therefore perhaps unsurprising that PCs are able to encode predictive and feedback signals of both movement and task performance, the latter associated with cognitive involvement (Popa and Ebner 2019). Such findings suggest that cognitive processing contributes to cerebellar-mediated learning by providing information about whether or not an action was successful

(Popa and Ebner 2019), just as reward signals can reinforce accuracy of goal-directed movements as described in Sect. 5.3.3.

7 Summary

The cerebellum is traditionally thought of as a brain structure with a highly regular cytoarchitecture, concerned primarily with motor control. However, we now know that there are additional complexities to cerebellar circuits, including recurrent loops both within the cerebellum and between the cerebellum and other brain regions, as well as systematic anatomical and physiological differences between cerebellar regions that likely relate to regional specialization of function. Nevertheless, a feature that may be common to all cerebellar circuits is the computation of differences between expected and actual outcomes of behavior, in all its different forms—enabling the cerebellum to regulate a wide variety of motor and non-motor functions via general principles applied to different brain networks. A key challenge for future research is to understand how the physiology of the cerebellum enables it to function as a universal prediction machine.

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Cerebellar Biochemistry/Pharmacology

Takahiro Seki

Abstract Cerebellar Purkinje cells (PCs) are localized in the cerebellar cortex and are characterized by highly developed dendrites, which receive different inputs from the parallel fibers of cerebellar granule cells, climbing fibers of inferior olive neurons in the medulla, and other cerebellar interneurons. PCs are the sole output neurons of the cerebellar cortex and are crucial for cerebellar functions. PC degeneration and morphological changes in PC dendrites are frequently observed in postmortem patients and mouse models of various cerebellar ataxias. Therefore, the factors that regulate the survival and morphology of PCs may be associated with the pathogenesis and progression of cerebellar ataxia. In this chapter, I summarize the interactions between PCs and other cerebellar cells that affect the survival and morphology of cerebellar PCs. Furthermore, numerous studies have revealed that intracellular protein degradation systems contribute to the maintenance of protein homeostasis and are essential for neuronal survival and function retention. Therefore, I also outline the role of protein degradation systems in the regulation of the survival and function of cerebellar neurons. Lastly, I briefly describe the endogenous modulators that affect the survival and morphology of cerebellar PCs.

Keywords Purkinje cells · Parallel fibers · Climbing fibers · Ubiquitin-proteasome system · Autophagy-lysosome pathways

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_3

1 Introduction

The cerebellum was traditionally considered to be involved in motor control and motor learning. However, increasing evidence has indicated that it also participates in cognitive processing and emotional control (Schmahmann and Caplan 2006), with different cerebellar areas involved in regulating motor and cognitive functions. The anterior vermis of the cerebellum mainly regulates motor function, while the posterior vermis and lateral hemispheres of the cerebellum control cognitive and emotional functions (Stoodley et al. 2016). Despite the regional differences in cerebellar function, the cerebellum comprises a uniform structure of layers. The cerebellar cortex consists of three layers: the molecular layer, Purkinje cell (PC) layer, and granule cell layer (Fig. 1). Among the cerebellar cortex neurons, PCs play a central role in regulating cerebellar function (Cerminara et al. 2015; Kalinichenko and Pushchin 2018; van der Heijden and Sillitoe 2021). Somata of PCs are lined up in the PC layer, while highly developed dendrites of PCs are projected onto the molecular layer. Numerous somata of granule cells are found in the granule cell layer. Granule cells receive inputs from mossy fibers originating from neurons in various regions, including the pons, medulla, midbrain, and spinal cord, and

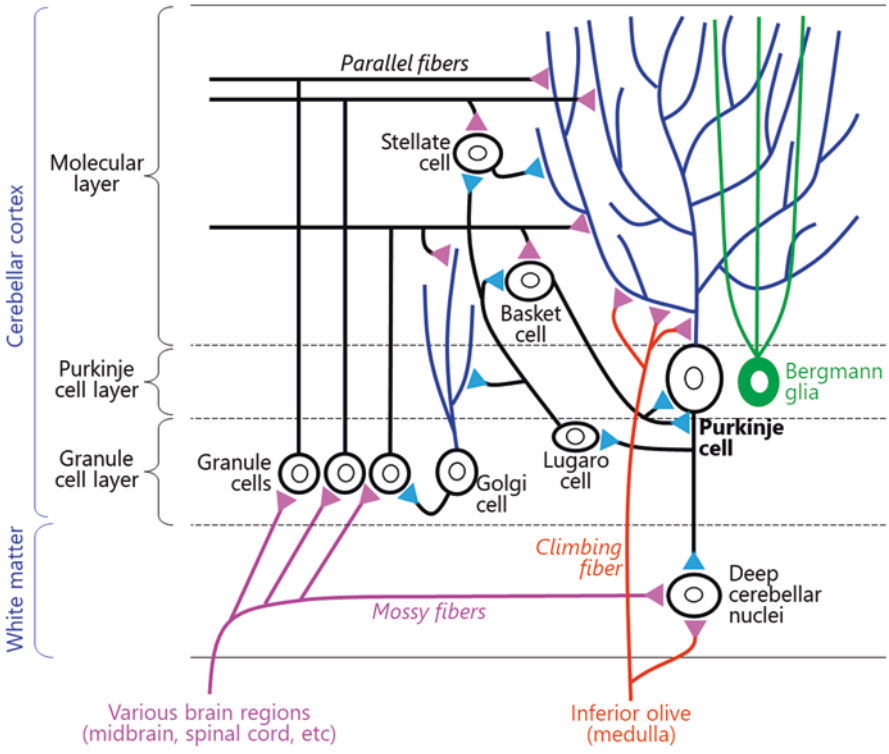

Fig. 1 Schematic illustration of layer structure, cell types, and neural connections in the cerebellum

innervate the dendrites of PCs as parallel fibers. Dendrites of PCs also receive inputs from climbing fibers originating from the inferior olive neurons in the medulla (Kalinichenko and Pushchin 2018). However, each PC is innervated by a single climbing fiber at the proximal dendrites, whereas each PC receives inputs from parallel fibers of many granule cells at the distal dendrites. The parallel and climbing fibers form excitatory glutamatergic synapses with PC dendrites. The activities of PCs are also regulated by γ-aminobutyric acid (GABA)ergic interneurons (stellate and basket cells) localized in the molecular cell layer (Kalinichenko and Pushchin 2018). There are several inhibitory interneurons localized in the granule layer, including Golgi and Lugaro cells. Golgi cells regulate excitatory inputs from mossy fibers to granule cells. Lugaro cells regulate other inhibitory interneurons.

Although the cerebellar cortex receives inputs from mossy and climbing fibers originating from other brain regions, PCs are the sole output neurons of the cerebellar cortex that go on to innervate the neurons in deep cerebellar nuclei (DCN) (Fig. 1) (D'Angelo et al. 2011). Therefore, PCs are crucial for cerebellar function. Functional or morphological aberrations or degeneration of PCs is frequently observed in patients with ataxia and ataxic animal models (Klockgether et al. 2019; Koeppen 2018). Most spontaneous ataxic mutant animals exhibit degeneration or dysfunction of the PCs (Cendelin 2014). Genetic analyses of these spontaneous ataxic mutant animals have revealed factors that regulate the dendritic morphology and survival of PCs. The regulation of dendritic development has also been investigated using organotypic or dissociated cerebellar cultures. In this chapter, I focus on the interactions between PCs and other cerebellar cells, protein degradation systems, and endogenous modulators that affect the morphology, survival, and function of PCs.

Natural mutant ataxic model animals, treatment-induced ataxic model animals, and abbreviations that I describe in this chapter are listed in Tables 1, 2, and 3, respectively.

Magenta and cyan triangles indicate excitatory (glutamatergic) and inhibitory (GABAergic) inputs. Black and blue lines indicate axons and dendrites of cerebellar neurons, respectively.

2 Interactions Between Purkinje Cells and Other Cerebellar Cells

2.1 Parallel and Climbing Fibers (Granule Cells and Inferior Olive Neurons)

Glutamatergic input from parallel and climbing fibers is related to the synaptic plasticity (formation of long-term potentiation [LTP] and long-term depression [LTD]) of PCs as well as motor coordination and motor learning (Ito 2001; Vogt and Canepari 2010). Additionally, input variations from these fibers affect the dendritic morphology and survival of PCs. This aspect has been researched in various animal models, such

Table 1 Natural mutant ataxic animals described in this chapter

Name	Species	Gene	Phenotype	Reference
Weaver	Mouse	Missense mutation of *Kcnj6* gene encoding GIRK2	Loss of granule cells	Sotelo (1975)
Reeler	Mouse	Deletion mutation of *Reln* gene encoding reelin	Loss of granule cells	Heckroth et al. (1989)
Shaker	Rat	Missense mutation of *Atp2b3* gene encoding plasma membrane calcium pump isoform 3 (PMCA3)	Loss of Purkinje cells	Figueroa et al. (2016)
Pingu	Mouse	Missense mutation of *Kana2* gene encoding Kv1.2	Loss of function of Kv1.2	Xie et al. (2010)
Long Evans Shaker	Rat	Deletion mutation of *Mbp* gene	Demyelination of Purkinje cells	Barron et al. (2018)
Tambaleante	Mouse	Missense mutation of *Herc1* gene	Loss of Purkinje cells	Wassef et al. (1987)
Purkinje cell degeneration (pcd)	Mouse	Deletion mutation of *Nna1* gene	Loss of Purkinje cells	Fernandez-Gonzalez et al. (2002)
Staggerer	Mouse	Deletion mutation of *Rora* gene encoding RORα	Impaired development of Purkinje cells	Hamilton et al. (1996)
Lucher	Mouse	Missense mutation of *Grid2* gene encoding GluRδ2	Loss of Purkinje cells	Zuo et al. (1997)
Hotfoot	Mouse	Deletion mutation of *Grid2* gene encoding GluRδ2	Impairment of synaptic formation in PCs	Lalouette et al. (1998)
Wistar Kyoto	Rat	Missense mutation of *Abcg5* gene encoding ATP-binding cassette transporter protein G5 (ABCG5)	Cerebellar shrinkage (Reduced expression of CBS in the cerebellum)	Nagasawa et al. (2015)

Table 2 Treatment-induced ataxic animals described in this chapter

Name	Species	Phenotype	Reference
X-Irradiation	Mouse	Loss of granule cells	Sotelo and Dusart (2009)
3-Acetylpyridine	Rat	Loss of inferior olive neurons and climbing fibers	Heckroth et al. (1989)
Ibogaine	Rat	Glutamate excitotoxicity of Purkinje cells from climbing fibers	Xu et al. (2000)
Ethanol	Rat, mouse	Transient cerebellar ataxia	Saeed Dar (2015)

Table 3 List of abbreviations

Abbreviation	Full spell
AIS	Axonal initial segment
ALP	Autophagy-lysosome pathway
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
3-AP	3-Acetylpyridine
Atg7	Autophagy-related gene 7
BDNF	Brain-derived neurotrophic factor
CBS	Cystathionine β-synthase
cGMP	Cyclic guanosine monophosphate
CHIP	C-terminus of Hsc70-interacting protein
CMA	Chaperone-mediated autophagy
CSE	Cystathionine γ-lyase
DAO	D-Amino acid oxidase
DCN	Deep cerebellar nuclei
EA	Episodic ataxia
EAAT	Excitatory amino acid transporter
ERK	Extracellular signal-regulated kinase
GABA	γ-Aminobutyric acid
GIRK2	G-protein-regulated inward-rectifier potassium channel 2
GLAST	Glutamate/aspartate transporter
GluRδ2	Glutamate receptor δ2
GLT1	Glial glutamate transporter 1
HERC1	HECT and RLD domain containing E3 ubiquitin protein ligase family member 1
Hsc70	Heat shock cognate protein 70 kDa
LAMP2A	Lysosome-associated membrane protein 2A
LTD	Long-term depression
LTP	Long-term potentiation
MA	Macroautophagy
mA	Microautophagy
MBP	Myelin basic protein
3MP	3-Mercaptopyruvate
MS	Multiple sclerosis
3MST	3-Mercaptopyruvate sulfur transferase
MVB	Multivesicular body
MNDA	*N*-methyl-D-aspartate
nNOS	Neural nitric oxide synthase
NO	Nitric oxide
NPC	Niemann-Pick disease type C
PC	Purkinje cell
pcd	*Purkinje cell degeneration*
PI31	Proteasomal inhibitor of 31 kDa
PKC	Protein kinase C
PKG	Protein kinase G

(continued)

Table 3 (continued)

Abbreviation	Full spell
RORα	Receptor-related orphan receptor α
RyR1	Type 1 ryanodine receptor
SCA	Spinocerebellar ataxia
tbl	*Tambaleante*
TH	Thyroid hormone
TRE	TH response element
TRH	Thyrotropin-releasing hormone
UPS	Ubiquitin-proteasome system

as *weaver* mice, which are natural mutant mice with a missense mutation in G-protein-regulated inward-rectifier potassium channel 2 (GIRK2) (Hess 1996). GIRK2 is mainly expressed in granule cells in the cerebellum. The *weaver* mutation of GIRK2 hampers inwardly rectifying K^+ current (Surmeier et al. 1996), leading to the loss of granule cells is observed in early cerebellar development (Sotelo 1975). Despite the loss of parallel fibers derived from granule cells, PC dendrites are developed in *weaver* mice. However, the length of the dendrites is smaller, and the orientation of the dendrites is disturbed (Sotelo 1975). Similar findings have been reported in other mutant mice, such as *reeler* mice, in which granule cells also degenerate at the developmental stage (Heckroth et al. 1989), and in agranular rats, which are postnatally X-irradiated to deplete granule cells (Sotelo and Dusart 2009). The *weaver* and *reeler* mice also show a reduction in PCs in the cerebellum (Heckroth et al. 1989; Sotelo 1975). Additionally, PC degeneration is also observed in *shaker* rats which have a missense mutation of *Atp2b3* gene encoding plasma membrane calcium pump isoform 3 (PMCA3) (Figueroa et al. 2016). A missense mutation of PMCA3 is also identified in patients with X-linked congenital cerebellar ataxia (Zanni et al. 2012). PMCA3 is expressed in the presynaptic terminals of parallel fibers that project to PCs (Burette and Weinberg 2007). PMCA3 has a role in maintenance of intracellular Ca^{2+} homeostasis to reduce cytosolic Ca^{2+} after the transient Ca^{2+} increase (Brini and Carafoli 2009). A missense mutation of PMCA3 found in patients delays the clearance of cytosolic Ca^{2+} (Zanni et al. 2012), which would result in dysregulation of neurotransmitter release from parallel fibers. In vitro studies using purified PCs from embryonic mouse cerebella have shown that granule cells are essential for the complete development of PC dendrites and survival of PCs in dissociated cultures (Carlos et al. 1994). Although glial cells and neurons from other regions partially help in the survival of purified PCs, these cells do not have adequately developed dendrites. These findings indicate that inputs from the parallel fibers of granule cells are necessary for the normal development of PC dendrites and PC survival.

The innervation of PCs by the climbing fibers of inferior olive neurons has been investigated using 3-acetylpyridine (3-AP), a toxin of inferior olive neurons. 3-AP is frequently used to develop drug-induced ataxic rat models (Llinás et al. 1975). 3-AP functions as a metabolic antagonist and decreases nicotinamide, leading to the inhibition of nicotinamide nucleotide dinucleotide (NAD^+)-dependent reactions. The administration of 3-AP followed by nicotinamide triggers the selective

degeneration of inferior olive neurons (Llinás et al. 1975). The anti-ataxic effect of taltirelin, a thyrotropin-releasing hormone that is clinically used as a therapeutic agent for spinocerebellar ataxia (SCA), has been evaluated in 3-AP-induced ataxic rats (Kinoshita et al. 1995). Several reports have indicated that 3-AP triggers the neurodegeneration of PCs (Chong et al. 2020; Mahmoudi et al. 2019). Additionally, 3-AP accelerates PC degeneration in *shaker* mutant rats (Tolbert and Clark 2000). These findings indicate that climbing fibers from inferior olive neurons contribute to PC survival. However, other studies have demonstrated that the loss of climbing fibers induced by 3-AP does not affect the survival of PCs but affects their electrophysiological properties, leading to an ataxic phenotype (Kaffashian et al. 2011; Rossi et al. 1991). Although the role of climbing fibers in the survival of PCs is controversial, the importance of these inputs for motor coordination is established.

Ibogaine is an indole alkaloid extracted from *Tabernanthe iboga* that triggers the degeneration of PCs in the rat cerebellum (Xu et al. 2000). However, this neurotoxicity is not caused by a direct effect of ibogaine on PCs but is mediated by the climbing fibers, as demonstrated by the elimination of this toxicity by 3-AP-induced ablation of the climbing fibers (O'Hearn and Molliver 1997). Since at the synapses between PCs and climbing fibers are glutamatergic, glutamate excitotoxicity is involved in ibogaine-triggered degeneration of PCs. Glutamate-induced neurodegeneration of PCs is called dark cell degeneration and is mediated by the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and not *N*-methyl-D-aspartate (NMDA) receptors (Garthwaite and Garthwaite 1991; Strahlendorf et al. 2003). However, ibogaine-triggered degeneration of PCs is not inhibited but enhanced by treatment with an AMPA receptor antagonist (O'Hearn and Molliver 2004). Although PCs are classically considered not to express NMDA receptors, functional NMDA receptors are reported to be postsynaptically expressed at synapses between PCs and climbing fibers (Piochon et al. 2010). These findings suggest that glutamate toxicity through the activation of NMDA receptors at PC-climbing fiber synapses could cause ibogaine-triggered neurodegeneration of PCs. Thus, excessive excitation of climbing fibers triggers the neurodegeneration of PCs.

Figure 2 summarizes molecular mechanisms in PC degeneration and PC dendritic shrinkage by the dysfunctions of parallel and climbing fibers.

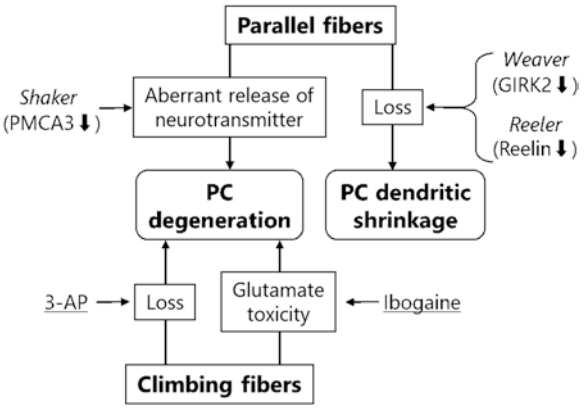

Fig. 2 Molecular mechanisms in PC degeneration and PC dendritic shrinkage by the dysfunctions of parallel and climbing fibers

2.2 Basket Cells

GABAergic basket cells innervate the PC somata and axonal initial segments (AIS) of PCs (Fig. 1). Nerve terminals from basket cells surround the AIS and form characteristic structures called pinceau (Somogyi and Hámori 1976). The voltage-gated potassium channel α-subunits (Kv1.1 and Kv1.2) are concentrated in these pinceau structures (Wang et al. 1994). Mutations in these subunits induce ataxic phenotypes. Missense mutations in Kv1.1 are associated with episodic ataxia 1 (EA1), which is characterized by stress-induced and recurrent attacks of ataxia (Browne et al. 1994). EA1 model mice, which carry the heterozygous missense mutation (V408A) in Kv1.1, show stress-induced motor impairment (Herson et al. 2003). This ataxic phenotype is ameliorated by treatment with acetazolamide, a clinical therapeutic agent used for episodic ataxia (Zasorin et al. 1983). Electrophysiological studies showed increased GABAergic input to PCs in EA1 model mice (Herson et al. 2003), a phenotype similar to that of Kv1.1-deficient mice (Zhang et al. 1999). *Pingu* mice that are generated by treatment with a chemical mutagen (*N*-ethyl-*N*-nitrosourea) carry a missense mutation in Kv1.2 and present with chronic motor incoordination (Xie et al. 2010). The motor incoordination in *Pingu* mice is ameliorated by treatment with acetazolamide, similar to that observed in EA1 model mice (Herson et al. 2003). Although electrophysiological properties are not affected in *Pingu* mice, the missense mutation in Kv1.2 makes this protein unstable (Xie et al. 2010). These findings indicate that loss of function of Kv1.1 and Kv1.2 caused by missense mutations in these proteins leads to the development of the ataxic phenotype. Since α-dendrotoxin, an inhibitor of Kv1.1 and Kv1.2, enhances inhibitory synaptic inputs from basket cells to PCs (Southan and Robertson 1998), loss of these channels would trigger hyperexcitation of basket cells and excessive inhibition of PCs. Acetazolamide-mediated intracellular alkalinization may reduce excitability of the basket cells (Herson et al. 2003). These findings suggest that inhibitory input from basket cells to PCs is crucial for motor coordination.

2.3 Glial Cells

Bergmann glia are radial astrocytes that are associated with PCs (Bellamy 2006). Their somata are closely localized to the PC somata in the PC layer and project processes to the molecular layer (Fig. 1). The radial structure of Bergmann glia contributes to layer formation during the developmental stage (Xu et al. 2013). In the adult cerebellum, Bergmann glia express glial glutamate transporters (including glutamate/aspartate transporter [GLAST]/excitatory amino acid transporter 1 [EAAT1] and glial glutamate transporter 1 [GLT1]/EAAT2) at the radial processes and regulate glutamatergic neurotransmission at the climbing fiber-PC and parallel fiber-PC synapses (Takayasu et al. 2009). GLAST is specifically expressed in Bergman glia in the cerebellum. Therefore, Bergmann glia regulate the activity and

synaptic plasticity of PCs through the modulation of synaptic glutamate uptake. Early activation of Bergmann glia, which is characterized by the elevation of glial fibrillary acidic protein, is frequently observed in animal models of cerebellar ataxia, including various types of SCAs (Cvetanovic et al. 2015; Seki et al. 2018a). In particular, a reduction in GLAST is observed in SCA mouse models (Cvetanovic 2015; Noma et al. 2012). The reduction in GLAST may hamper synaptic glutamate uptake, increase glutamate concentration at the synaptic cleft, and trigger glutamate excitotoxicity in PCs, leading to PC degeneration in SCA. Moreover, GLAST-deficient mice show motor impairment and increased susceptibility to cerebellar injury (Watase et al. 1998), while the expression of SCA7-causing mutant protein in Bergmann glia leads to non-cell-autonomous degeneration of PCs and the ataxic phenotype through reduction in GLAST (Custer et al. 2006).

Furthermore, reduction in Bergmann glia themselves is observed in the cerebella of patients with SCA1 and SCA1 mouse models (Shiwaku et al. 2013). Moreover, a model of astrogliosis that was generated by chronic optogenetic activation of Bergmann glia showed downregulation of GLAST and degeneration of PCs (Shuvaev et al. 2021). These results suggest that Bergmann glia activation and reduction of Bergmann glia contribute to the pathogenesis and progression of cerebellar ataxia through the dysregulation of glutamate uptake and induction of non-cell autonomous PC degeneration.

Early inflammatory activation of microglia is commonly observed in animal models of various neurodegenerative diseases (Wolf et al. 2017). In line with these findings, microglial activation may be involved in neurodegeneration. Similarly, microglial activation is observed in the early stages of cerebellar ataxia in ataxic model animals (Ferro et al. 2019). Inhibition of microglial activation via the genetic ablation of myeloid differentiation factor 88, which is involved in the inflammatory activation of microglia (Esen and Kielian 2006), ameliorates PC degeneration and motor dysfunction in SCA6 model mice (Aikawa et al. 2015). Additionally, lipopolysaccharide-triggered microglial activation in the cerebellum leads to degeneration of PCs and motor impairment (Hong et al. 2020). However, this treatment also activates astrocytes, including the Bergmann glia. These findings indicate that the inflammatory activation of microglia directly or indirectly triggers cerebellar neurodegeneration in SCA.

Cerebellar ataxia commonly occurs in multiple sclerosis (MS), which is caused by the progressive demyelination of neurons in the central nervous system (Wilkins 2017). Loss of PCs and abnormal morphology of PC axons are observed in the cerebella of patients with MS (Redondo et al. 2015). Furthermore, a decrease in the myelination of PC axons results in reduced GABAergic inputs to DCN neurons and the hyperactivation of DCN neurons in *Long Evans Shaker* rats (Barron et al. 2018), in which myelin basic protein (MBP) is genetically deleted (Delaney et al. 1995). MBP is expressed in oligodendrocytes and is a major constituent of the myelin sheath in the central nervous system (Rumsby and Walker 1980). These results suggest that oligodendrocytes regulate the survival and synaptic transmission of PCs via the myelination of PC axons.

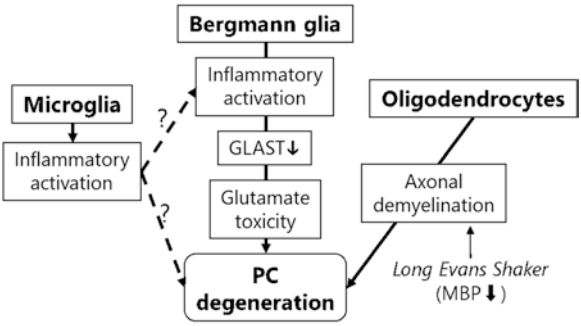

Fig. 3 Molecular mechanisms in PC degeneration by the aberrant regulation of glial cells

Furthermore, two different groups independently revealed that transcriptional changes in oligodendrocyte-related genes are present in SCA3 model mice (Haas et al. 2021; Ramani et al. 2017). Hass et al. also demonstrated that MBP and oligodendrocyte transcription factor 2, which are expressed in oligodendrocytes, are reduced in the cerebella of patients with SCA3 (Haas et al. 2021). These findings strongly suggest that functional alterations in oligodendrocytes contribute to the pathogenesis of SCA.

Figure 3 summarizes molecular mechanisms in PC degeneration by the aberrant regulation of glial cells.

3 Importance of Protein Degradation Systems in Cerebellar Purkinje Cells

3.1 Classification of Protein Degradation Systems

Protein degradation systems contribute to maintaining intracellular protein homeostasis in cells. Intracellular protein degradation systems are mainly divided into the ubiquitin-proteasome system (UPS) and autophagy-lysosome pathway (ALP) (Fig. 4) (Dikic 2017; Wang and Le 2019). In the UPS, substrate proteins are selectively polyubiquitinated by various E3 ubiquitin ligases. The polyubiquitinated proteins are then delivered to the proteasome, a large protein complex containing multiple proteases, where ubiquitinated substrates are degraded (Fig. 4a) (Hegde and Upadhya 2011). The ALP consists of three pathways that deliver substrates to lysosomes: macroautophagy (MA), microautophagy (mA), and chaperone-mediated autophagy (CMA) (Fig. 4b) (Haspel and Choi 2011), among which MA has been the most widely studied (Mizushima and Levine 2020). In MA, substrate proteins are surrounded by isolation membranes, incorporated into autophagosomes, and delivered to lysosomes via the fusion of autophagosomes with lysosomes. In comparison to MA, the physiological roles of mA and CMA are not well understood (Tekirdag and Cuervo 2018). The molecular mechanisms of mA and CMA in mammalian cells has remained unclear until recently. The heat shock cognate protein

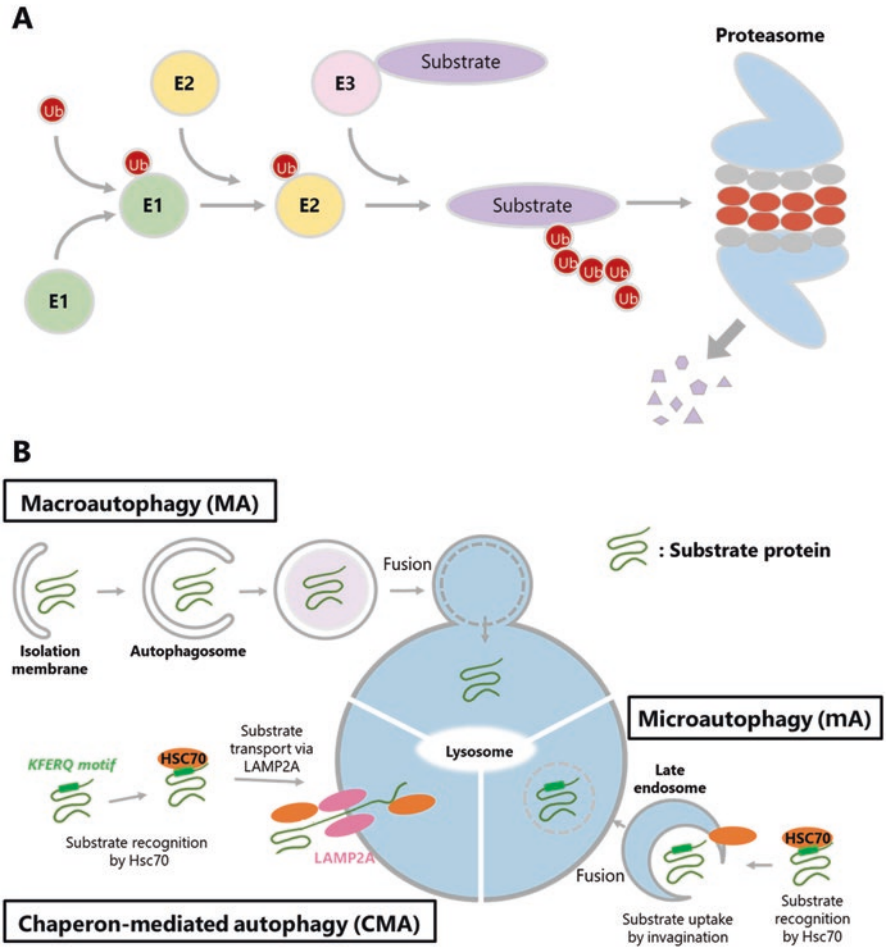

Fig. 4 Schematic illustrations of ubiquitin-proteasome system (**a**) and autophagy-lysosome pathway (**b**). Ub: ubiquitin, E1: ubiquitin activation enzyme, E2: ubiquitin conjugating enzyme, E3: E3 ubiquitin ligase

70 kDa (Hsc70), a molecular chaperone, commonly recognizes substrates, which carry specific pentapeptide sequences called KFERQ motifs (Kirchner et al. 2019), for mA and CMA. In mA, substrates are delivered into late endosomes via the invagination of endosomal membranes, resulting in the formation of multivesicular bodies (MVBs). Next, MVBs deliver the intravesicular substrates for lysosomal degradation by fusing with lysosomes. In CMA, substrates are directly transferred into lysosomes through the lysosomal translocon formed by the oligomerization of lysosome-associated membrane protein 2A (LAMP2A) on the lysosomal membrane.

Various studies have demonstrated that decline in intracellular protein degradation is related to the pathogenesis of multiple diseases via the disturbance of protein homeostasis (Hanna et al. 2019; Mizushima and Levine 2020). In particular,

non-dividing neuronal cells are strongly dependent on protein degradation systems for protein homeostasis maintenance (Malgaroli et al. 2006). Impairment of protein degradation systems has been frequently observed in patients and animal models of neurodegenerative diseases (Ciechanover and Kwon 2015; Douglas and Dillin 2010). In support with these findings, neuron-specific knockout of proteasome- or MA-related proteins have been found to induce phenotypes similar to neurodegenerative diseases, including accumulation of misfolded proteins and neurodegeneration (Hara et al. 2006; Komatsu et al. 2006; Tashiro et al. 2012). Moreover, age-related decline in protein degradation may be related to the age-related pathogenesis of various neurodegenerative diseases (Douglas and Dillin 2010).

3.2 Ubiquitin-Proteasome System in Cerebellar Purkinje Cells

Tambaleante (*tbl*) mutant mice show progressive degeneration of cerebellar PCs and a severe ataxic phenotype inherited in an autosomal recessive manner (Wassef et al. 1987). This phenotype is caused by a missense mutation in the HECT and RLD domain containing E3 ubiquitin protein ligase family member 1 (HERC1), a ubiquitin ligase protein (Mashimo et al. 2009). Transgenic rescue of wild-type HERC1 (Mashimo et al. 2009) indicates that the loss of function of HERC1 causes this cerebellar phenotype. Indeed, a nonsense mutation of *HERC1* gene is identified in a patient with megalencephaly accompanied with cerebellar atrophy (Nguyen et al. 2016). HERC1 regulates extracellular signal-regulated kinase (ERK) signaling via the degradation of C-RAF that phosphorylates and activates ERK (Schneider et al. 2018). Yang et al. reported that the upregulation of C-RAF impairs synapse formation in cultured cerebellar granule cells (Yang et al. 2013). Therefore, an increase in C-RAF that is caused by the loss of function of HERC1 might reduce synaptic inputs from parallel fiber to PCs, leading to the degeneration of PCs. Additionally, axonal degeneration and loss of PCs are triggered by the PC-specific knockout of proteasomal inhibitor of 31 kDa (PI31), a proteasome-binding protein (Minis et al. 2019). Although PI31 was first identified as an inhibitor of proteasomes in vitro, it has been reported to serve as an adapter protein for the axonal transport of proteasomes and to help proteasomal protein degradation in axon terminals in vivo (Liu et al. 2019). Furthermore, the axonal swelling and accumulation of ubiquitinated proteins at the axonal terminals around DCN neurons precede degeneration of PI31-knockout PCs (Minis et al. 2019). These findings suggest that the UPS plays a role in the maintenance of axon function and survival of PCs.

Aggregates of mutant proteins are observed in the cerebellar neurons of postmortem patients and animal models of several SCAs (Seidel et al. 2012). Moreover, it was found that proteasomal components are frequently recruited to these aggregates in cellular and mouse models of SCAs (Chai et al. 1999; Cummings et al. 1998; Seki et al. 2007). Additionally, genetic mutations in the C-terminus of Hsc70-interacting protein (CHIP), another ubiquitin ligase, have been identified as

etiologies of SCA48 and spinocerebellar ataxia, autosomal recessive 16 (SCAR16) (Genis et al. 2018; Shi et al. 2013). Since CHIP mediates the ubiquitination for the degradation of aggregate-prone proteins, it markedly contributes to protein quality control by the UPS (Jiang et al. 2001). Kanack et al. recently demonstrated that various CHIP missense mutations found in SCAR16 commonly destabilize CHIP (Kanack et al. 2018). Therefore, a decline in protein quality control by the UPS may be involved in the pathogenesis of cerebellar ataxia. However, age-related decline of proteasomal activity is not observed in the mouse cerebellum, which is in contrast to that found in other brain regions, including the cerebral cortex, hippocampus, and spinal cord (Keller et al. 2000). Therefore, it remains unclear whether UPS impairment is the main etiology of cerebellar ataxia.

3.3 Autophagy-Lysosome Pathways in Cerebellar Purkinje Cells

Neurodegeneration in the cerebellum is frequently observed in lysosomal storage disorders (LSDs), which are caused by a deficiency of lysosomal enzymes (Platt et al. 2012). Prominent degeneration of cerebellar PCs and severe motor impairment are observed in patients and animal models of Niemann-Pick disease type C (NPC), which is caused by the genetic loss of NPC1 or NPC2 that leads to the characteristic accumulation of cholesterol in lysosomes (Tang et al. 2010). LSDs are accompanied by decreased activity of ALP-mediated protein degradation (Settembre et al. 2008). Therefore, these results suggest that PCs are vulnerable to ALP impairment.

The importance of MA (one of the pathways in the ALP) in PCs was first demonstrated in mice with PC-specific knockout of autophagy-related gene 7 (Atg7), an MA-related protein (Komatsu et al. 2007). Swelling of axon terminals and slight loss of PCs were observed at postnatal day 56 of the Atg7-knockout mice, while dendritic arbors and motor function were not affected. These results suggest that MA is more strongly related to the maintenance of axon function than to the regulation of survival and dendritic morphology of PCs. Additionally, MA impairment is observed in cellular and animal models of SCAs (Alves et al. 2014; Onofre et al. 2016), suggesting MA impairment might be commonly related to the pathogenesis of cerebellar ataxia.

A recent report revealed that PC-specific knockdown of cathepsin D, a lysosomal proteolytic enzyme involved in all three pathways of the ALP, resulted in the greater impairment of the survival of PCs than PC-specific knockdown of Atg7 (Koike et al. 2017). Therefore, CMA or mA may have greater contribution to the survival of PCs than MA. In line with this finding, miRNA-mediated knockdown of LAMP2A, a CMA-related protein, in cerebellar neurons triggers neurodegeneration of PCs and other cerebellar neurons and progressive motor impairment, whereas knockdown of tumor susceptibility gene 101 protein, an mA-related protein, does not affect motor function in mice (Sato et al. 2021). Additionally, several SCA-causing proteins

commonly impair CMA (Seki et al. 2012, 2018a), indicating that CMA plays a pivotal role in the survival of cerebellar PCs and that CMA impairment is involved in the common pathogenesis of cerebellar ataxia.

Several studies have revealed that SCA-causing proteins commonly induce shrinkage of PC dendrites in cerebellar primary cultures (Irie et al. 2014; Ohta et al. 2021; Seki et al. 2009, 2018a). A similar phenotype has also been reported in cultured PCs differentiated from SCA6 patient-derived induced pluripotent stem cells (Ishida et al. 2016). Dendritic shrinkage of PCs precedes PC degeneration and is related to the onset of motor dysfunction in several SCA mouse models (Shakkottai et al. 2011; Watanave et al. 2019). Therefore, these findings suggest dendritic shrinkage of cultured PCs might be an in vitro phenotype commonly observed in various types of SCAs. Furthermore, similar dendritic shrinkage of PCs was observed in cultured PCs treated with a lysosomal inhibitor at low concentration (Seki et al. 2018a), suggesting that the ALP regulates the morphology of PC dendrites.

As described above, the impairment of ALP disrupts the survival and morphology of PCs. Conversely, several reports indicates that an excessive MA contributes to PC neurodegeneration. A transient ischemia induces MA activation and PC degeneration in juvenile rats (Au et al. 2015). This PC degeneration is inhibited by the siRNA-mediated knockdown of Atg7, an MA-related protein (Au et al. 2015), suggesting the involvement of the excessive MA in PC degeneration. An excessive MA is also observed in PCs of *Purkinje cell degeneration* (*pcd*) mice prior to PC neurodegeneration (Chakrabarti et al. 2009). *Pcd* mice exhibit adult-onset degeneration of PCs that is caused by the deletion mutation of *Nna1* gene (Fernandez-Gonzalez et al. 2002), a zinc carboxypeptidase localized in the nucleus and cytoplasm (Harris et al. 2000). Although there is no evidence indicating the relationship between Nna1 and MA, Nna1 cleaves peptides generated from proteasome-mediated proteolysis to amino acids and is related to protein turnover (Berezniuk et al. 2010). Additionally, an enhancement of MA is also observed in *tbl* mice described above (Mashimo et al. 2009). HERC1 mediates the degradation of tuberous sclerosis complex 2, which negatively regulates the activity of mammalian target of rapamycin (mTOR) (Chong-Kopera et al. 2006). Since the inhibition of mTOR potently activates MA (Mizushima and Levine 2020), the elevation of TSC2 caused by the loss of function of HERC1 in *tbl* mice triggers MA activation. Motor training decreases MA activity and prevents PC degeneration (Fucà et al. 2017), supports the importance of MA activation in PC degeneration. Since many autophagosomes in PCs contain mitochondria in *pcd* mice (Chakrabarti et al. 2009), aberrant or enhanced mitophagy (MA-mediated degradation of mitochondria) might be involved in the PC degeneration triggered by MA activation. These findings suggest that the disturbance of ALP activity affects the morphology and survival of PCs and causes cerebellar ataxia.

Figure 5 summarizes molecular mechanisms in PC degeneration and PC axonal dysfunction by the dysregulation of UPS and ALP.

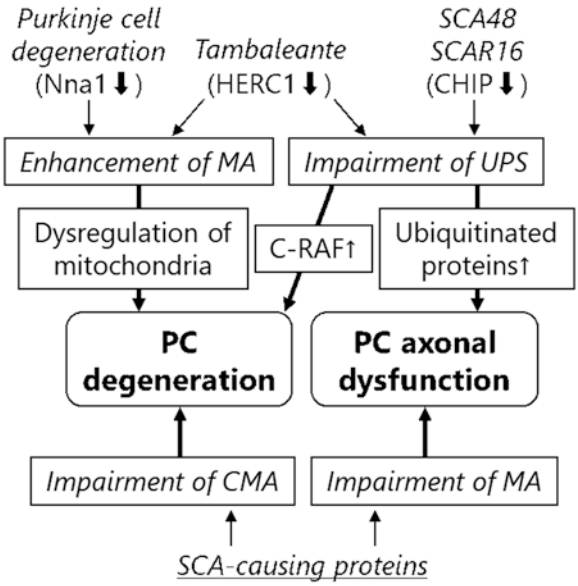

Fig. 5 Molecular mechanisms in PC degeneration and PC axonal dysfunction by the dysregulation of UPS and ALP

4 Endogenous Modulators of Purkinje Cells

4.1 Thyrotropin-Releasing Hormone (TRH)

Although TRH is a hypothalamic hormone that enhances the release of thyroid-stimulating hormone from the pituitary gland, it is also distributed in extra-hypothalamic brain regions, including the cerebellum, and may function as a neurotransmitter or neuromodulator (Shibusawa et al. 2008). Both TRH and its orally available analog, taltirelin, exert an anti-ataxic effect on 3-AP-induced ataxic rats and several types of natural mutant animals showing the ataxic phenotype (Kinoshita et al. 1995, 1998; Muroga et al. 1982; Nakamura et al. 2005). Therefore, these chemicals have been approved for the clinical treatment of cerebellar ataxia (Kinoshita et al. 1995; Sobue et al. 1983). However, the detailed molecular mechanism of the anti-ataxic effect of TRH has not been fully elucidated. Histological studies have revealed that the TRH receptor (type 2) is expressed in granule cells and interneurons of the cerebellar cortex but not in PCs (Sun et al. 2000), indicating that TRH has no direct effect on PCs. Moreover, NMDA receptors were found to mediate the anti-ataxic effect of taltirelin in 3-AP-treated rats (Kinoshita et al. 1998). Since the TRH receptor is also expressed in inferior olive neurons in the medulla (Sun et al. 2000), the anti-ataxic effect of TRH might be mediated by glutamate released from the climbing fibers. Recently, Watanave et al. reported that TRH is involved in motor learning (Watanave et al. 2018). Although cerebellar morphology is not affected, LTP at synapses between PCs and parallel fibers is diminished by TRH-knockout mice. Furthermore, treatment with TRH rescues motor

learning deficits and loss of LTP in TRH-knockout mice, and this rescue is mediated by nitric oxide (NO) and the subsequent generation of cyclic guanosine monophosphate (cGMP) (Watanave et al. 2018). Since neural NO synthase (nNOS) is mainly expressed in granule cells and interneurons of the cerebellum (Vincent and Kimura 1992), TRH may stimulate NO production in granule cells and interneurons, followed by transsynaptic activation of guanylate cyclase and increase in cGMP in PCs.

4.2 Thyroid Hormones (THs)

THs (3,3′,5-triiodothyronine [T3], and thyroxine [T4]) regulate neuronal migration, differentiation, and axonal myelination during the postnatal development of the cerebellum (Faustino and Ortiga-Carvalho 2014). The knockout of TH-related genes has proved the importance of THs in cerebellar development. Impaired development of PC dendrites and motor incoordination are observed in paired box gene 8-knockout mice, which are hypothyroid because of the malformation of the thyroid gland (Horn et al. 2013), and in mice lacking monocarboxylate transporter 8 and organic anion transporting polypeptide 1c1, which are involved in the transport of THs through the blood-brain barrier (Mayerl et al. 2014). THs bind to nuclear TH receptors (TRs) and enhance the expression of genes involved in cerebellar development, including nerve growth factor, brain-derived neurotrophic factor (*BDNF*), and retinoid receptor-related orphan receptor α (*RORα*) (Koibuchi and Iwasaki 2006).

Among them, RORα is abundant in cerebellar PCs and closely related to cerebellar development. *Staggerer* mice, a natural mutant in which the *RORα* gene is mutated (Hamilton et al. 1996), show tremor, motor incoordination, and impaired development of PC dendrites (Herrup and Mullen 1981; Sidman et al. 1962). Genetic deletion of RORα triggers phenotypes similar to those observed in *staggerer* mouse (Dussault et al. 1998), suggesting that loss of function of RORα impairs the development of cerebellar PCs in *staggerer* mice. A decrease in RORα in cerebellar PCs was also observed in the SCA model transgenic mice (Konno et al. 2014; Serra et al. 2006). Additionally, miRNA-mediated knockdown of RORα triggers atrophy of PC dendrites, decreased PC survival, and motor impairment in adult mice (Yasui et al. 2021). These reports suggest that RORα plays a pivotal role in the maintenance of dendritic morphology and survival of mature PCs as well as their development.

THs enhance TR-mediated transcription from promoters containing TH response elements (TREs) (Koibuchi and Iwasaki 2006). Although *RORα* is one of the target genes of THs, it also interacts with TR and enhances TH-mediated transcription from promoters containing TRE (Koibuchi et al. 1999). Additionally, BDNF is not upregulated during cerebellar development in *staggerer* mice (Qiu et al. 2007). Therefore, these findings demonstrate that RORα is crucial for TH-mediated development of the cerebellum.

4.3 Glutamate Receptor δ2 (GluRδ2) and D-Serine

Lurcher mice are natural mutant mice characterized by an ataxic phenotype and loss of multiple neurons around the cerebellum, including PCs, granule cells, interneurons in the molecular layer, and inferior olive neurons in the medulla (Phillips 1960; Zanjani et al. 2006). The *Lurcher* phenotype is caused by missense mutation in GluRδ2 (Zuo et al. 1997), which is an orphan receptor similar to ionotropic glutamate receptors and is predominantly expressed at the dendritic spines of cerebellar PCs (Yuzaki 2004). The mutation found in *Lurcher* mice constitutively activates GluRδ2, leading to the apoptosis of PCs during the postnatal development of the cerebellum (Zuo et al. 1997). However, *hotfoot* mice, whose GluRδ2 genes are deleted, and GluRδ2-knockout mice only show a mild ataxic phenotype (Kurihara et al. 1997; Lalouette et al. 1998). Loss of GluRδ2 does not trigger degeneration of PCs but it impairs the formation and stabilization of synapses between PCs and parallel fibers and the monoinnervation of PCs by climbing fibers (Yuzaki 2004). Therefore, GluRδ2 regulates synaptic formation and the survival of PCs during postnatal development of the cerebellum. Additionally, an antibody against the extracellular domain of GluRδ2 impaired the induction of LTD in cerebellar slices. Moreover, this antibody also triggered a transient ataxic phenotype in adult mice when it was injected into subarachnoid supracerebellar space (Yuzaki 2004). These findings suggest that GluRδ2 also plays an essential role in the regulation of synaptic plasticity in PCs of the mature cerebellum.

Kakegawa et al. identified D-serine as an endogenous ligand of GluRδ2 (Kakegawa et al. 2011). Exogenous treatment with D-serine reduces AMPA receptor expression at PC-parallel fiber synapses through GluRδ2-induced endocytosis of AMPA receptors (Kakegawa et al. 2011). D-serine is generated from L-serine by serine racemase, mainly in astrocytes, and acts as a co-agonist of NMDA receptors through the glycine-binding site (Schell et al. 1997). Moreover, the reduction in D-serine in patients with schizophrenia (Hashimoto et al. 2003) suggest that D-serine acts as an endogenous gliotransmitter that regulates NMDA receptor function. Although serine racemase is expressed in the Bergmann glia of cerebellum (Wolosker et al. 1999), the amount of D-serine in the adult cerebellum is lower than that in the forebrain (Miyoshi et al. 2012; Schell et al. 1997). This is due to the cerebellar-specific expression of D-amino acid oxidase (DAO), a metabolic enzyme of D-serine (Kim et al. 2019; Shibuya et al. 2013). Since the expression of DAO gradually increases after birth and peaks at 4 weeks of age (Shibuya et al. 2013), D-serine exists in the cerebellum only during the early postnatal period. D-serine colocalizes with NMDA receptors at the PC dendrites in the immature cerebellum (Schell et al. 1997). Furthermore, NMDA receptors are reported to be present in the cerebellum only during the developmental period (Garthwaite et al. 1987), and these receptors are important for the synaptic elimination of PCs from climbing fibers to establish monoinnervation to PCs by climbing fibers (Green et al. 1992). Lastly, D-serine contributes to the induction of LTD via GluRδ2 in the immature cerebellum (Kakegawa et al. 2011). These results suggest that D-serine participates in synaptic formation through both NMDA receptors and GluRδ2 during cerebellar development.

4.4 Nitric Oxide (NO)

NO synthase (NOS) is more abundant in the cerebellum than in other brain regions (Förstermann et al. 1990). Therefore, NO may play a crucial role in cerebellar function. In line with this idea, motor coordination deficits have been reported in mice lacking nNOS (Kriegsfeld et al. 1999), indicating that nNOS is involved in cerebellar function. nNOS is mainly expressed in granule cells and interneurons in the molecular layer but not in PCs (Vincent and Kimura 1992). However, NO is related to both LTP and LTD induction in PCs (Daniel et al. 1998; Kakizawa et al. 2012). Therefore, NO derived from the axonal terminals of parallel fibers and interneurons transmits and regulates intracellular signals in PCs. In LTD induction, NO activates guanylate cyclase and increases intracellular cGMP, followed by activation of protein kinase G (PKG). Activation of both PKG, along with the activation of protein kinase C (PKC) by the stimulation of metabotropic glutamate receptor 1 (mGluR1), then induces LTD at the synapses between PCs and parallel fibers (Daniel et al. 1998). In LTP induction, NO activates type 1 ryanodine receptors (RyR1) via its S-nitrosylation and induces Ca^{2+} release from the endoplasmic reticulum (Kakizawa et al. 2012).

The nNOS-derived NO also regulates the dendritic morphology of PCs. Knockout of nNOS increases dendrite thickness, reduces mature dendritic spines, and decreases the expression of mGluR1 in mature PCs (Tellios et al. 2020). Although vesicular glutamate transporter 1, which is localized at the presynaptic terminals of parallel fibers, is not affected in nNOS-knockout mice, synaptic responses from parallel fibers are reduced because of the decrease in mGluR1, which is involved in the synaptic responses from parallel fibers (Yamasaki et al. 2021). Moreover, synaptic terminals from climbing fibers are increased in nNOS-knockout mice (Tellios et al. 2020). These findings indicate that nNOS-derived NO modulates the balance of neural inputs between parallel and climbing fibers in PCs. Additionally, nNOS is specifically involved in the survival of primary cultured PCs, while other types of NOS are not (Oldreive et al. 2012).

A decrease in mGluR1 signaling is frequently observed in animals and patients with SCA (Yamasaki et al. 2021). Additionally, a positive allosteric modulator of mGluR1 improved the motor performance of SCA1 model mice (Notartomaso et al. 2013). In contrast, the receptor activity is enhanced by missense mutations of mGluR1 that are identified in patients with SCA44 (Watson et al. 2017). Therefore, the disturbance of mGluR1 signaling is related to the pathogenesis of cerebellar ataxia. nNOS-derived NO might have an important role in regulating mGluR1 signaling in PCs during the pathogenesis of cerebellar ataxia.

As described above, NO plays an essential role in regulating synaptic transmission, morphology, and survival under physiological conditions. This is supported by the findings that show NOS activity and nitrotyrosine levels are strongly increased in the cerebellum of *Lurcher* mice (McFarland et al. 2007). Since reactive nitrogen is toxic to primary cultured PCs (Oldreive et al. 2012), oxidative stress by excessive NO may contribute to the degeneration of PCs in *Lurcher* mice. However,

deficiency of nNOS does not affect PC degeneration and nitrotyrosine formation (McFarland et al. 2007), indicating that other types of NOS trigger excessive NO production in *Lurcher* mice. Since reactive microglia accumulate in the cerebellum of *Lurcher* mice (Cairns et al. 2017), inducible NOS (iNOS) in reactive microglia would be responsible for excessive NO production in *Lurcher* mice. Microglial activation is also frequently observed in various animal models of cerebellar ataxia as described in Sect. 2.3. Therefore, excessive NO generated from iNOS may be involved in the pathogenic mechanisms of cerebellar ataxia.

Acute ethanol intake triggers transient cerebellar ataxia, characterized by loss of motor coordination and dysarthria, in human and animal models (Saeed Dar 2015). Ethanol rapidly decreases nNOS amount in rat cerebellum (Auta et al. 2020). Inactivation of nNOS by ethanol would lead to excessive activation of PCs and GABA-mediated suppression of neurons in DCN (Saeed Dar 2015). Taken together, both excess and deficiency of NO in the cerebellum impairs PC functions and triggers ataxic phenotype.

4.5 Hydrogen Sulfide and D-Cysteine

Hydrogen sulfide has recently been investigated as an endogenous gasotransmitter similar to NO (Kimura 2021). It modifies protein function via the persulfidation of cysteine residues and regulates various neural functions, including LTP. Additionally, hydrogen sulfide enhances the persulfidation of free L-cysteine and glutathione and increases anti-oxidative and neuroprotective activities (Paul and Snyder 2018). Hydrogen sulfide is endogenously generated from L-cysteine through four different pathways: cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), cysteine aminotransferase/3-mercaptopyruvate sulfurtransferase (3MST) (Kamoun 2004), and cysteinyl-tRNA synthetase (Akaike et al. 2017). Decrease in these enzymes and hydrogen sulfide production are associated with several neurodegenerative diseases, including Parkinson's disease, Huntington's disease, and Alzheimer's disease (Paul and Snyder 2018). Conversely, the administration of hydrogen sulfide or its donors provides a protective effect in the experimental models of neurodegenerative diseases (Kida et al. 2011; Xie et al. 2013).

Among the hydrogen sulfide-generating enzymes, CBS is related to cerebellar morphology and function. It is more abundantly expressed in the cerebellum than in other brain regions (Enokido et al. 2005). Studies have reported that patients with CBS deficiency present with severe progressive polymyoclonus and ataxia (Awaad et al. 1995). Additionally, the cerebellum is markedly smaller in CBS-knockout mice (Enokido et al. 2005). Similarly, the *Wistar Kyoto* rat, an animal model of depression, shows reduced expression of CBS along with a smaller cerebellum (Nagasawa et al. 2015). These findings suggest that CBS is involved in the development and function of the cerebellum. As for the other hydrogen sulfide-generating enzymes, CSE shows reduced expression in patients with SCA3, while

overexpression of CSE rescues the ataxic phenotype of SCA3 model *Drosophila* (Snijder et al. 2015).

Shibuya et al. revealed a novel pathway for the generation of hydrogen sulfide from D-cysteine (Shibuya et al. 2013). In this pathway, D-cysteine is converted to 3-mercaptopyruvate (3MP) by DAO, followed by the generation of hydrogen sulfide from 3MP by 3MST. As described in Sect. 4.3, DAO is selectively expressed in the cerebellum (Kim et al. 2019). Hydrogen sulfide is more effectively and selectively generated from D-cysteine in the cerebellum than in other brain regions (Snijder et al. 2015). Therefore, D-cysteine could be a novel hydrogen sulfide donor that is selective to the cerebellum. D-cysteine enhances the dendritic development of PCs via the production of hydrogen sulfide (Seki et al. 2018b). Both DAO and 3MST are reported to be expressed in astrocytes (Ono et al. 2009; Zhao et al. 2013). Since CBS and CSE are also expressed in Bergmann glia (Enokido et al. 2005; Snijder et al. 2015), hydrogen sulfide in the cerebellum is mainly generated in Bergmann glia and transactivates PCs, leading to the enhancement of dendritic development. D-cysteine ameliorates dendritic shrinkage in primary cultured PCs expressing several types of SCA-causing proteins (Ohta et al. 2021). Additionally, it suppresses the onset of motor dysfunction, neurodegeneration, and glial activation in SCA1 model mice (Ohta et al. 2021). These results indicate the potential of hydrogen sulfide supplementation as a therapeutic strategy for cerebellar ataxia, along with D-cysteine as a preventive drug for SCAs.

Among the various D-amino acids, only D-serine and D-aspartate are generated and functional in mammalian tissues (Genchi 2017). Earlier, D-cysteine was neither considered present nor produced in mammals. However, Semenza et al. recently reported that D-cysteine is endogenously generated by serine racemase in the mouse brain, where brain D-cysteine concentration is highest during the embryonic period (Semenza et al. 2021). During this period, D-cysteine regulates the proliferation and differentiation of neural precursor cells (Semenza et al. 2021). D-cysteine is also present in the adult mouse brain, especially in the forebrain regions, where DAO is not expressed (Ono et al. 2009). Since serine racemase is expressed in Bergmann glia (Wolosker et al. 1999), hydrogen sulfide might be constitutively generated in Bergmann glia from D-cysteine, which is converted from L-cysteine by serine racemase, and contributes to the physiological functions in the cerebellum.

Figure 6 summarizes molecular mechanisms how endogenous factors regulate survival, morphology, and synaptic plasticity of PCs.

5 Concluding Remarks

Increasing evidence about the regulation of survival and morphology of cerebellar PCs provides the possible common mechanisms for cerebellar ataxia: (1) dysregulation of synaptic inputs from other neurons, (2) inflammatory glial activation, (3) dysregulation of ALP-mediated proteolysis, (4) dysregulation of NO production.

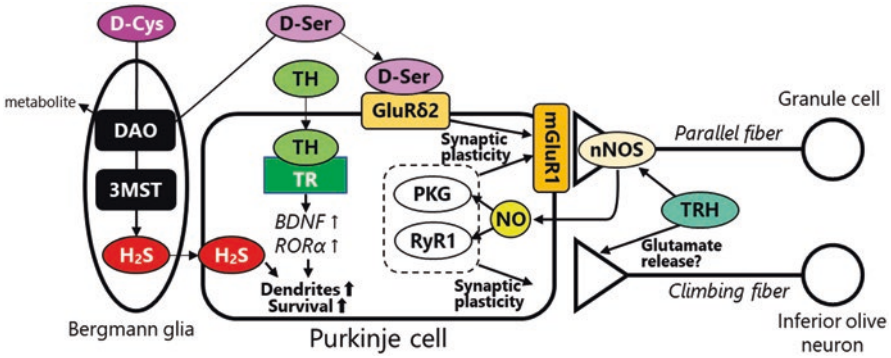

Fig. 6 Molecular mechanisms how endogenous factors regulate survival, morphology, and synaptic plasticity of PCs

The establishment of the methods to regulate these mechanisms will contribute to developing novel therapeutic strategies for cerebellar ataxia.

Acknowledgment I would like to thank Editage (www.editage.com) for English language editing.

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Genetics of Dominant Ataxias

Ashraf Yahia and Giovanni Stevanin

Abstract Autosomal dominant cerebellar ataxia (ADCA) accounts for more than fifty inherited neurological diseases caused by various underlying mechanisms leading to progressive or intermittent phenotypes. Most ADCAs develop due to alterations in signal transduction, ion homeostasis, or pathological DNA expansions. Yet, other pathological mechanisms can directly or indirectly contribute to the development of these diseases. Initially, ADCA diagnosis was dominated by linkage and nucleotide repeat expansion analyses. However, the advent of next-generation sequencing has enhanced ADCA diagnosis, including the discovery of novel forms due to nucleotide repeat expansions and conventional mutations, with limited reliable genotype–phenotype correlations within the major ADCA subgroups. The developments in ADCA diagnosis have outpaced the discovery of effective treatments and biomarkers for these diseases, particularly the progressive neurodegenerative subtypes. Multiple clinical trials are, however, underway with some promising results but there are still many challenges to overcome.

Keywords Cerebellar ataxia · Nucleotide repeat expansion · Channelopathies · Genetic diagnosis · Polyglutamine expansion

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_4

Abbreviations

ADCA	Autosomal dominant cerebellar ataxia
ADCADN	Autosomal dominant cerebellar ataxia, deafness, and narcolepsy
CAPOS	Cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss
CNV	Copy number variation
DHA	Docosahexaenoic acid
DRPLA	Dentatorubral-pallidoluysian atrophy
EA	Episodic ataxia
EEAT1	Excitatory amino acid transporter-1
EGFR	Epidermal growth factor receptor
ER	Endoplasmic reticulum
NGS	Next-generation sequencing
NMDA	*N*-methyl-D-aspartate
NPTX1	Neuronal pentraxin 1 protein/gene
RAN	Repeat-associated non-AUG
SCA	Spinocerebellar ataxia
SCA42ND	SCA42 with neurodevelopmental deficits
SPTBN2	Spectrin beta non-erythrocytic-2 protein
UTR	Untranslated region
WES	Whole exome sequencing
WGS	Whole genome sequencing

1 Introduction

Ataxia refers to a neurological condition characterized by poorly coordinated movements. Autosomal dominant forms of cerebellar ataxias (ADCAs) encompass >50 clinically and genetically heterogeneous disorders (Klockgether et al. 2019; Giunti et al. 2020). Their estimated world-wide prevalence is 2.7/100,000 in most populations (Ruano et al. 2014) but in some regions can reach higher values because of founder effects, such as in the province of Holguin in Cuba (47.9/100,000) (Velázquez-Pérez et al. 2020) or in the islands of the Azores (39/100,000) (De Araújo et al. 2016).

Most ADCAs fall within the spinocerebellar ataxia (SCA) subgroup, followed in frequency by episodic ataxias (EAs). SCAs usually have a progressive course due to degeneration of the cerebellum and/or its connections. These involvements are reflected in their core presentation of gait ataxia and incoordination, dysarthria, and oculomotor problems (Klockgether et al. 2019; Müller 2021). In more complex SCA forms, other structures of the nervous system degenerate as well, particularly the brainstem (Sullivan et al. 2019; Müller 2021). At the other end of the spectrum, EAs are characterized by intermittent attacks of cerebellar dysfunction of variable

duration, sometimes accompanied by other paroxysmal neurological conditions, such as epilepsy, migraine, and dystonia (Giunti et al. 2020; Harvey et al. 2021).

Pathologically, most ADCA patients suffer from the consequences of nucleotide repeat expansions or, to a lesser extent, channelopathies. However, other disease mechanisms are directly implicated in the pathogenesis of some ADCA subtypes. These include abnormal gene expression or alterations of lipids metabolism, endolysosomal/autophagy pathway, mitochondria, and energy metabolism (Matilla-Dueñas et al. 2014; Klockgether et al. 2019; Sullivan et al. 2019). The successive identification of seven nucleotide repeat expansion-linked dominant ataxias in the nineties, and a few more in recent years, and their involvement in almost half of all patients, have for a long time focused attention on these particular mutations (Durr 2010; Krygier and Mazurkiewicz-Bełdzińska 2021). The report of point mutations in dominant ataxias is more recent (Krygier and Mazurkiewicz-Bełdzińska 2021). Table 1 lists the different ADCA subtypes and their mutational mechanisms.

2 Genetic Diagnosis

Linkage analysis and candidate gene analyses have dominated the discovery of novel ADCA subtypes for decades (Yahia and Stevanin 2021). However, during the past decade, next-generation sequencing (NGS)-based approaches have been taking over. NGS is becoming more and more efficient due to the ongoing improvement in its associated algorithms that enable the detection of a wide spectrum of variations, including DNA repeat expansions and deletions (Chintalaphani et al. 2021). Of particular interest are the recent advances in long-read genomic sequencing (e.g., Oxford Nanopore and PacBio technologies) and optical mapping methods (e.g., Bionano), as they can unravel disease-causing structural variants that may explain part of the missing heritability in ataxias (Yahia and Stevanin 2021).

In the clinics and other large-scale settings, except in patients with episodic presentation of ataxia, most ADCA patients are initially screened for repeat expansions using PCR-based approaches in view of the frequency of the repeat-associated subtypes (Cagnoli et al. 2018; Kang et al. 2019; Velázquez-Pérez et al. 2020; Bogdanova-Mihaylova et al. 2021; Riso et al. 2021). The detection rate for repeat expansion screening depends on several factors including, among others, the origin of the studied patients, the age at disease onset, the velocity of disease progression, the type and number of genes screened, and the presence of an anticipation of the age at onset. For example, in a Cuban cohort with dominant ataxia, the detection rate for repeat expansion screening was 90.8% (881/970) and 87% of the screened cases had a repeat expansion in *ATXN2* (Velázquez-Pérez et al. 2020). The detection rates in two large Asian cohorts were 62.5% (932/1489) in a Chinese cohort and 52% (131/265) in a Thai cohort, with SCA3 as the most common subtype (Boonkongchuen et al. 2014; Choubtum et al. 2015; Chen et al. 2018). In a smaller Japanese cohort (34/52), SCA6 was the most common genetic entity with an overall 65% diagnostic yield (Sakakibara et al. 2017). On the other hand, the detection rate of these SCAs

Table 1 List of autosomal dominant cerebellar ataxia subtypes, their causative genes, and mutational mechanisms

Disease	Gene	OMIM[a]	Mutational mechanism	Reference
SCA1	*ATXN1*	# 164400	CAG/polyglutamine expansion	Banfi et al. (1994)
SCA2	*ATXN2*	# 183090	CAG/polyglutamine expansion	Pulst et al. (1996)
SCA3	*ATXN3*	# 109150	CAG/polyglutamine expansion	Kawaguchi et al. (1994)
SCA4		% 600223		Hellenbroich et al. (2003)
SCA5	*SPTBN2*	# 600224	Point mutation	Ikeda et al. (2006) and Perkins et al. (2016)
SCA6	*CACNA1A*	# 183086	CAG/polyglutamine expansion	Zhuchenko et al. (1997) and Du et al. (2013)
EA2		# 108500	Point mutation	Ophoff et al. (1996)
Early onset progressive ataxia			Point mutation	Yue et al. (1997) and Tonelli et al. (2006)
SCA7	*ATXN7*	# 164500	CAG/polyglutamine expansion	David et al. (1997)
SCA8	*ATXN8OS*	# 608768	Untranslated and CAG/polyglutamine expansions	Koob et al. (1999)
	ATXN8			Moseley et al. (2006)
SCA9		% 612876		Higgins et al. (1997)
SCA10	*ATXN10*	# 603516	Untranslated repeat expansion	Matsuura et al. (2000)
SCA11	*TTBK2*	# 604432	Point mutation	Houlden et al. (2007) and Bowie and Goetz (2020)
SCA12	*PPP2R2B*	# 604326	Untranslated repeat expansion	Holmes et al. (1999)
SCA13	*KCNC3*	# 605259	Point mutation	Waters et al. (2006) and Khare et al. (2017)
SCA14	*PRKCG*	# 605361	Point mutation	Chen et al. (2003) and Wong et al. (2018)
SCA15/16	*ITPR1*	# 606658	CNV and rarely point mutation	Van De Leemput et al. (2007)
SCA29		# 117360	Point mutation	Huang et al. (2012)
SCA17	*TBP*	# 607136	CAG/polyglutamine expansion	Koide et al. (1999)
SCA18		% 607458		Brkanac et al. (2002)
SCA19/ SCA22	*KCND3*	# 607346	Point mutation	Lee et al. (2012) and Hsiao et al. (2019)
SCA20		# 608687		Knight et al. (2008)
SCA21	*TMEM240*	# 607454	Point mutation	Delplanque et al. (2014) and Seki et al. (2018)

(continued)

Table 1 (continued)

Disease	Gene	OMIM[a]	Mutational mechanism	Reference
SCA23	*PDYN*	# 610245	Point mutation	Bakalkin et al. (2010) and Smeets et al. (2015, 2020)
SCA25	*PNPT1*	# 608703	Point mutation	Stevanin et al. (2004) and Barbier et al. (2022)
SCA26	*EEF2*	# 609306	Point mutation	Hekman et al. (2012)
SCA27 EA-like	*FGF14*	# 609307	Point mutation	Van Swieten et al. (2003), Tempia et al. (2015), and Piarroux et al. (2020)
SCA28	*AFG3L2*	# 610246	Point mutation, CNV	Di Bella et al. (2010) and Pareek and Pallanck (2020)
SCA30		% 613371		Storey et al. (2009)
SCA31	*BEAN1*	# 117210	Untranslated repeat expansion	Sato et al. (2009)
SCA34	*ELOVL4*	# 133190	Point mutation	Cadieux-Dion et al. (2014) and Sherry et al. (2017)
SCA35	*TGM6*	# 613908	Point mutation	Wang et al. (2010) and Tripathy et al. (2017)
SCA36	*NOP56*	# 614153	Untranslated repeat expansion	Kobayashi et al. (2011)
SCA37	*DAB1*	# 615945	Untranslated repeat expansion	Seixas et al. (2017)
SCA38	*ELOVL5*	# 615957	Point mutation	Di Gregorio et al. (2014)
SCA39			Large 11q duplication	Johnson et al. (2015)
SCA40	*CCDC88C*	# 616053	Point mutation	Tsoi et al. (2014)
SCA41	*TRPC3*	# 616410	Point mutation	Fogel et al. (2015)
SCA42	*CACNA1G*	# 616795	Point mutation	Coutelier et al. (2015a)
SCA42ND		# 618087		Chemin et al. (2018)
SCA43	*MME*	# 617018	Point mutation	Depondt et al. (2016)
SCA44	*GRM1*	# 617691	Point mutation	Watson et al. (2017)
SCA45	*FAT2*	# 617769	Point mutation	Nibbeling et al. (2017)
SCA46	*PLD3*	# 617770	Point mutation	Nibbeling et al. (2017)
SCA47	*PUM1*	# 617931	Point mutation	Gennarino et al. (2015, 2018)
SCA48	*STUB1*	# 618093	Point mutation	Genis et al. (2018)
SPAX1	*VAMP1*	# 108600	Point mutation	Bourassa et al. (2012)
GRID2	*GRID2*		Point mutation	Kumagai et al. (2014) and Coutelier et al. (2015b)
SCA50	*NPTX1*	# 620158	Point mutation	Coutelier et al. (2021)
KCNA2	*KCNA2*		Point mutation	Helbig et al. (2016)
DRPLA	*ATN1*	# 125370	CAG/polyglutamine expansion	Koide et al. (1994)
ADCADN	*DNMT1*	# 604121	Point mutation	Winkelmann et al. (2012) and Maresca et al. (2020)
CAPOS	*ATP1A3*	# 601338	Point mutation	Demos et al. (2014) and Tranebjærg et al. (2018)

(continued)

Table 1 (continued)

Disease	Gene	OMIM[a]	Mutational mechanism	Reference
EA1	*KCNA1*	# 160120	Point mutation	Browne et al. (1994) and Zhao et al. (2020)
EA3		% 606554		Steckley et al. (2001)
EA5	*CACNB4*	# 613855	Point mutation	Escayg et al. (2000) and Subramanyam et al. (2009)
EA6	*SLC1A3*	# 612656	Point mutation	Jen et al. (2005) and Winter et al. (2012)
EA7		% 611907		Kerber et al. (2007)
EA8		% 616055		Conroy et al. (2014)
EA9	*SCN2A*	# 618924	Point mutation	Liao et al. (2010) and Schwarz et al. (2016)
SCA49	*SAMD9L*	# 611170	Point mutation	Corral-Juan et al. (2022)

OMIM online Mendelian inheritance in man catalog (https://omim.org/), *SCA* spinocerebellar ataxia, *EA* episodic ataxia, *ADCADN* autosomal dominant cerebellar ataxia, deafness, and narcolepsy, *CAPOS* cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss
[a]OMIM entry number preceded by "%" indicates that the entry describes a confirmed phenotype for which the underlying molecular mechanism is not known, "#" indicates Mendelian phenotypes caused by known genes

was relatively low (13.8%, 11/80) in a cohort from Australia (Kang et al. 2019) and intermediate (42.8%, 12/28) in a cohort from Ireland (Bogdanova-Mihaylova et al. 2021). Generally, the relative frequencies of these SCAs is the result of past human migrations and the existence of founder effects in certain populations, such as SCA3 in Portugal and Asia (Martins et al. 2007; Martins and Sequeiros 2018), SCA2 in Cuba (Velázquez Pérez et al. 2009), SCA10 in South America (Almeida et al. 2009), SCA36 in Galicia (Spain) (Arias et al. 2017), and SCA12 in India (Bahl et al. 2005). Overall SCA3 is the major form in most countries but there are exceptions, e.g., Cuba and Italy (Klockgether et al. 2019).

Most of the time, because of their higher frequencies, only SCAs due to coding CAG repeat expansions are analyzed, but the diagnostic yield can be further enhanced by screening more genes for non-coding DNA repeat expansions (Choubtum et al. 2015; Chen et al. 2018) and enhanced even more using NGS-based approaches in negative cases to search for conventional variants (Galatolo et al. 2021). An NGS panel-based approach achieved a genetic diagnosis in 14.3% (59/412) of ADCA patients with negative results on DNA repeat expansion screening (Coutelier et al. 2017). A similar percentage, 13.5% (13/96), was obtained in another cohort after negative DNA repeat expansion screening (Nibbeling et al. 2017). The use of NGS-based genome-wide approaches, whole exome sequencing (WES) and whole genome sequencing (WGS), further enhance diagnostic success, with the potential for discovering novel causative genes or known genes in overlapping syndromes (Nibbeling et al. 2017; Kang et al. 2019; Ngo et al. 2020; Yahia and Stevanin 2021). This last point is illustrated by the recent identification of

heterozygous variants by WGS in *PNPT1* as responsible for SCA25 (Barbier et al. 2022), while this gene was previously reported in an autosomal recessive multisystem disorder with mitochondrial dysfunction (MIM#614932). Indeed, WGS is emerging as a promising first-tier test in rare diseases in general (Turro et al. 2020). Recent evidence showed its high sensitivity and specificity in detecting repeat expansions (Ibañez et al. 2022), advocating for its integration in routine ADCA diagnosis, possibly as the near future gold-standard genetic test. A limiting factor is the availability and economic feasibility of WGS which differ between countries. If WGS sequencing is not feasible, the choice of genetic tests for ADCA diagnosis is dictated by: tests' availability, patients' origin as discussed earlier, and the clinical context. An example for how clinical context could prioritize the diagnostic approach is that while screening for repeats expansion is advised in patients with progressive adult-onset ADCA (De Silva et al. 2019), NGS-based approaches (targeted gene panels and WES) or array genotyping are preferred in childhood-onset ADCA with negative family history and even in adult patient with very low progression rate (Brandsma et al. 2019).

3 ADCA Pathological Mechanisms

3.1 Abnormal Repeat Expansions

To date, 13 ADCAs are known to be associated with abnormal expansion of DNA repeats (Krygier and Mazurkiewicz-Bełdzińska 2021). Abnormal CAG repeats are located in protein-coding regions in seven ADCAs, SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, and dentatorubral-pallidoluysian atrophy (DRPLA), known collectively as CAG/polyglutamine SCAs (Banfi et al. 1994; Kawaguchi et al. 1994; Koide et al. 1994, 1999; Pulst et al. 1996; David et al. 1997; Zhuchenko et al. 1997). In addition, in SCA8, an abnormal CTG expansion is transcribed in both directions; in the sense strand it is located in the 3′ UTR of the gene, while in the anti-sense strand it is translated as CAG repeats and then into polyglutamine-expanded peptides when mutated (Koob et al. 1999; Moseley et al. 2006). In SCA12, the abnormal CAG repeat expansion is located in the 5′ untranslated region of the *PPP2R2B* gene (Holmes et al. 1999). In four other SCAs, the abnormal expansions are located in introns: SCA10, SCA31, SCA36, and SCA37 (Matsuura et al. 2000; Sato et al. 2009; Kobayashi et al. 2011; Seixas et al. 2017). Generally, when the disease-causing expansions are located outside coding exons, they tend to be much larger than those located in coding exons (Klockgether et al. 2019). Both exonic and intronic expansions are dynamic and their sizes vary between patients from the same family (Klockgether et al. 2019), objectified as somatic and germline instability. The presence of nucleotide interruptions in the repeat sequence may, however, stabilize the sequence and, consequently, instability is then rare in some cases, such as in SCA17. The repeat size affects the severity and age at onset of the disease in

many SCA subtypes; this contributes to the phenomenon of anticipation, more particularly in polyglutamine SCAs (Klockgether et al. 2019). In addition to the size of the pathological allele, normal alleles of some dominant ataxia genes, or variants of DNA repair genes influence the age at onset and disease severity (Du Montcel et al. 2014; Bettencourt et al. 2016).

The pathophysiology of polyglutamine SCAs differs depending on the functions and the interactions of the native proteins but they share a general mechanistic theme (Malik et al. 2021). The altered proteins in polyglutamine SCAs are directly or indirectly toxic to the cells (Klockgether et al. 2019; Malik et al. 2021). Direct toxicity to the cell comes from the altered proteins forming toxic compounds, losing their own functions, or sequestering other proteins beneficial to the cell (Klockgether et al. 2019; Malik et al. 2021). In addition, abnormal repeats can be translated in all six frames and both sense and antisense, the so-called repeat associated non-AUG (RAN) translation, which reinforces the toxic function hypothesis although this has not been demonstrated in all these pathologies (Zu et al. 2011). Indirect toxicity comes as a consequence of the direct toxicity by placing a continuous burden on the transcriptional and translational machinery in the cell (Klockgether et al. 2019; Niewiadomska-Cimicka et al. 2020). For example, most of the genes causing polyglutamine-SCAs encode proteins vital for regulating gene expression, which may explain part of the commonalities observed (Niewiadomska-Cimicka et al. 2020). For these reasons, multiple systems are affected in the cells and one of the most promising therapeutic strategy nowadays seems to reduce the expression of the pathological proteins at the RNA level using RNA interference or antisense oligonucleotides, before the toxic properties surpass the cell plasticity (Brooker et al. 2021).

Intronic repeats of SCAs cause cellular toxicity by sequestering RNA-binding proteins, affecting epigenomic processes such as RNA splicing and gene expression or forming toxic RNA aggregates (White et al. 2012; Seixas et al. 2017; Klockgether et al. 2019; Malik et al. 2021). RAN translation has also been implicated in the pathological process of some clinico-genetic entities. In the case of SCA12, overexpression of the *PPP2R2B* transcript in a size-dependent manner seems to be involved (O'Hearn et al. 2015).

3.2 Channelopathies and Alteration of the Signal Transduction Pathways

Neurons are particularly sensitive to alterations of the cellular functions ensuring their specific role in cell–cell communication. This includes channels important for ion homeostasis and action potential transmission, but also synaptic machinery dynamics (receptors, structural proteins, etc.), particularly glutamate signalling.

3.2.1 Specific Ion Channel Alterations

More than 140 genes encode voltage-gated ion channels (Alexander et al. 2019). Ion channels are important in the nervous system as they are pivotal for neuronal development, neurotransmission, action potentials, and other neuronal processes (Spillane et al. 2016; Smith and Walsh 2020). Genetic disorders of the ion channels can manifest prenatally, early in life, or after adulthood and can be episodic or progressive (Spillane et al. 2016; Noebels 2017; D'Adamo et al. 2020). Patients with channelopathies variably present with ataxia, epilepsy, migraine, brain malformations, and other neurological manifestations (Spillane et al. 2016; D'Adamo et al. 2020). The phenotypic spectrum of genetic channelopathies reflects the nature and vintage of the brain circuits where each particular channel acts (Noebels 2017; Smith and Walsh 2020). Channelopathies are caused by mutations that alter the channel function or mutations that affect its expression, post-translational modifications, trafficking/targeting, or assembly (Spillane et al. 2016; Noebels 2017). More rarely, some mutations disturb processes not directly related to the channel function, such as sequestering of other proteins (Khare et al. 2017). Mutations in voltage-gated and ligand-gated potassium, sodium, and calcium channels and transporters cause some subtypes of ADCA.

EA1, SCA13, and SCA19 are caused by mutations in the genes encoding the voltage-gated potassium channel subunits Kv1.1, Kv3.3, Kv4.3, respectively (Browne et al. 1994; Waters et al. 2006; Lee et al. 2012). Of note, SCA13 may not be a pure channelopathy as the p.R423H variant of the Kv3.3 channel indirectly sequesters the receptor of the epidermal growth factor (EGFR), possibly disturbing the EGFR-NOTCH morphogenetic signaling pathway, adding another layer of complexity to the SCA13 phenotype (Khare et al. 2017).

Abnormal calcium channels and transportation are direct causes of six subtypes of ADCAs. Mutations in the alpha Cav2.1 and alpha Cav3.1 subunits of the voltage-gated calcium channel cause EA2 and both SCA42 and SCA42 with neurodevelopmental deficits (SCA42ND), respectively, while mutations in the calcium channel voltage-dependent subunit beta-4 cause EA5 (Ophoff et al. 1996; Yue et al. 1997; Escayg et al. 2000; Coutelier et al. 2015a; Chemin et al. 2018). SCA15/SCA16 and SCA29 are caused by mutations in the *ITPR1* gene encoding inositol 1,4,5-triphosphate (IP3) receptor, an intracellular IP3-gated calcium channel that modulates intracellular calcium signaling (Van De Leemput et al. 2007; Huang et al. 2012).

Altered sodium trafficking causes only one ADCA type compared to potassium and calcium: EA9 is due to mutations in the *SCN2A* gene encoding the sodium voltage-gated channel alpha subunit-2 (Liao et al. 2010).

3.2.2 Alteration of the Synaptic Machinery

Channels and transporters regulating neurotransmitter function are also implicated in the pathogenesis of some ADCAs. Glutamate is the most abundant neurotransmitter in the brain and the one of the most involved in the pathogenesis of ADCAs

(Reiner and Levitz 2018). SCA41 is caused by mutations in the *TRPC3* gene, which encodes a nonselective cation channel highly expressed in Purkinje cells and linked to signaling pathways, including metabotropic glutamate receptor-dependent synaptic transmission (Fogel et al. 2015). Similarly, SCA44 and *GRID2*-linked ataxia are caused by mutations in *GRM1* and *GRID2,* encoding glutamate metabotropic receptor-1 and glutamate ionotropic receptor delta type subunit-2, respectively (Kumagai et al. 2014; Coutelier et al. 2015b; Watson et al. 2017). EA6 is caused by mutations in the *SLC1A3* gene encoding a glial glutamate transporter, the excitatory amino acid transporter-1 (EEAT1) (Jen et al. 2005). EEAT1 belongs to a family of transporters that regulate the concentration of neurotransmitters at excitatory glutamatergic synapses (Jen et al. 2005). Another member of this family, EEAT4, is indirectly implicated in the pathogenesis of SCA5, which is caused by mutations in the gene *SPTBN2* encoding spectrin beta non-erythrocytic-2 protein (SPTBN2) (Ikeda et al. 2006; Perkins et al. 2016). SPTBN2 stabilizes the Purkinje cell-specific glutamate transporter EAAT4 at the plasma membrane (Perkins et al. 2016). Recently, point mutations in the gene encoding neuronal pentraxin 1 (*NPTX1*) were shown to affect the protein stability and/or its secretion, which may affect its role in synapse dynamics (Coutelier et al. 2021). Finally, the *STUB1*-encoded protein is involved in *N*-methyl-D-aspartate (NMDA) receptor degradation (Ferreira et al. 2013) and accounts for recessive (SCAR16) and dominant (SCA48) ataxias (Shi et al. 2013; Genis et al. 2018), with the dominant form presenting at an earlier age and giving rise to a more severe phenotype (Roux et al. 2020).

3.3 Abnormal Gene Expression

Regulation of gene expression and other epigenetic processes is important for the developing and the mature brain (Starr 2019). Epigenetic regulators include chromatin and histone modifiers, mRNA regulators, and components of the transcription and the translation machinery (Starr 2019; Aygun and Bjornsson 2020). The autosomal dominant cerebellar ataxia, deafness, and narcolepsy syndrome (ADCADN) is caused by mutations in the DNA methyltransferase-1, a chromatin modifier encoded by the *DNMT1* gene (Winkelmann et al. 2012; Maresca et al. 2020). Similarly, pathogenic mutations in a Purkinje cell transcription regulator, α1ACT, contribute to SCA6 (Du et al. 2013). This transcript is encoded by *CACNA1A*, a bicistronic gene that is involved in EA2 through the other transcript (Ophoff et al. 1996; Du et al. 2013). Similarly, SCA47 is caused by mutations in the *PUM1* gene encoding a post-transcriptional regulator of gene expression that binds to the PUM recognition element in the 3′-untranslated region (UTR) of target mRNAs (Gennarino et al. 2015, 2018; Wolfe et al. 2020). Finally, impaired translation is also implicated in ADCA pathogenesis, as illustrated by SCA26, caused by mutations in the *EEF2* gene encoding eukaryotic translation elongation factor-2 (Hekman et al. 2012).

3.4 Disorders of Lipid Metabolism

Disorders of lipid metabolism are implicated in many neurodevelopmental and neurodegenerative conditions (Hussain et al. 2019; Darios et al. 2020; Grassi et al. 2020). Indeed, lipids are major molecular players in the nervous system. Three ADCA subtypes are caused primarily by defects in the metabolism of fatty acids and phospholipids. SCA34 and SCA38 are caused by mutations in the *ELOVL4* and *ELOVL5* genes, encoding elongation of very-long-chain-fatty-acids enzymes (Aldahmesh et al. 2011; Cadieux-Dion et al. 2014; Di Gregorio et al. 2014). Similarly, SCA46 is caused by mutations in the *PLD3* gene encoding the phospholipase D3 enzyme, important for the hydrolysis of phospholipids (Selvy et al. 2011; Nibbeling et al. 2017).

3.5 Other Mechanisms

Additional mechanisms contribute to the pathogenesis of ADCAs either directly or indirectly. Lysosomal and ER dysfunctions are directly implicated in the development of SCA21 and SCA35, respectively (Tripathy et al. 2017; Seki et al. 2018). *TMEM240* encodes a trans-membrane protein that induces lysosomal damage when mutated, leading to morphological changes in Purkinje cells of SCA21 animal models (Seki et al. 2018). Of note, the endolysosomal pathway also contributes to the pathogenesis of SCA6 (Unno et al. 2012). On the other hand, SCA35 involves aberrant activation of the unfolded protein response due to mutations in *TGM6*, which encodes the transglutaminase 6 protein (Tripathy et al. 2017). The point mutations in the neuronal pentraxin 1 gene (*NPTX1*) were shown to induce an ER stress through its retention, which may contribute partially to the disease (Coutelier et al. 2021), as in the case of some *KCND3* variants (Duarri et al. 2012). Finally, the gene encoding polyribonucleotide nucleotidyltransferase PNPase 1 (PNPT1) was found to be mutated in SCA25 patients, thereby linking cerebellar syndrome to mitochondrial DNA processing, a situation known in recessive ataxias (Barbier et al. 2022). Another important mitochondrial protein is a metalloprotease involved in maintenance of the mitochondrial proteome and which is composed of homodimers of AFG3L2 or heterodimers with paraplegin. Variants in the gene encoding AFG3L2 have been implicated in SCA28 (Di Bella et al. 2010), while variants in the gene encoding paraplegin are responsible for a frequent autosomal recessive spastic ataxia (Coarelli et al. 2019). Interestingly, heterozygous variants in the gene encoding paraplegin are suspected of contributing to late onset ataxia (Klebe et al. 2012).

4 Phenotype–Genotype Correlations

The cardinal difference between the two main ADCA subgroups is the progressive disease course in SCAs and the intermittent nature of EAs. Nevertheless, some EA patients develop persistent cerebellar features (Graves et al. 2014). On the other hand, there are very few distinctive phenotypic features that could predict the mutated gene within the different ADCA subgroups (Fig. 1). Dermal anomalies are recurrently seen in SCA34 (Bourque et al. 2018). Macular degeneration is still the best predictor of SCA7; however, since the clinical presentation depends on the size of the repeat, the age at examination and the presence of additional modifiers, this sign is not always present and has occasionally been reported in other forms such as SCA2 (Rufa et al. 2002; Michalik et al. 2004; Park et al. 2020). In addition, there are no distinctive ancillary or biochemical markers except serum docosahexaenoic acid (DHA) in SCA38 (Di Gregorio et al. 2014).

The constant reporting of new cases enlarges the phenotypic spectrum of each genetic entity so that they tend to overlap each other. This is well illustrated by the multiple clinico-genetic entities in which cerebellar ataxia and pyramidal features, also known as spastic ataxias, are associated. This clinical overlap also extends to other neurogenetic diseases; i.e., some cases with CAG repeat expansions in the

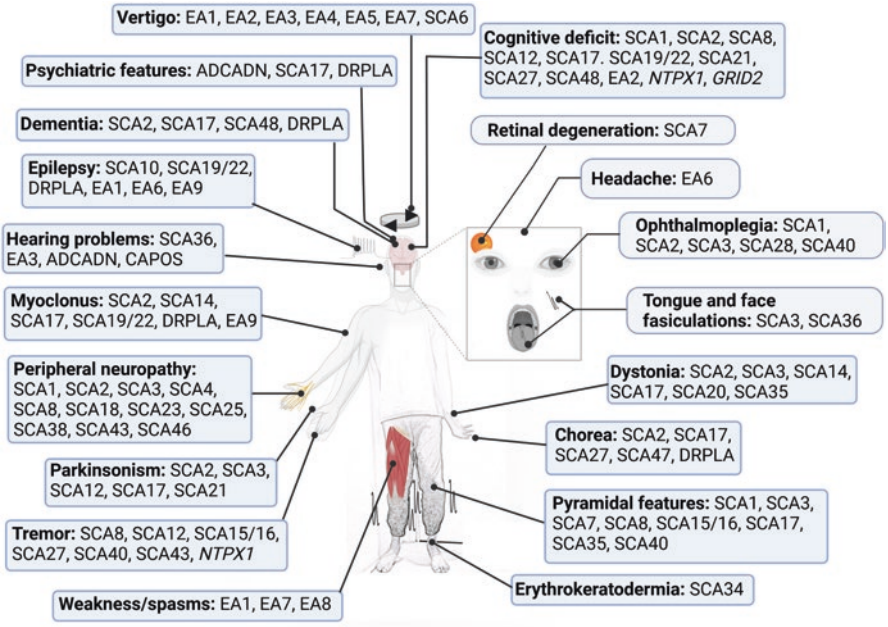

Fig. 1 Clinical signs that feature prominently with cerebellar ataxia in different autosomal dominant cerebellar ataxia subtypes. SCA spinocerebellar ataxia, EA episodic ataxia, ADCADN autosomal dominant cerebellar ataxia, deafness, and narcolepsy, CAPOS cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss

ATXN2 gene may have a cerebellar syndrome or a Parkinson-like phenotype (Charles et al. 2007), or be at risk for amyotrophic lateral sclerosis (Lattante et al. 2014) according to the CAA/CAG repeat size and its composition, a rare case of phenotype–genotype correlation in ADCA. Indeed, CAG repeat/polyglutamine SCAs are the only ADCA subgroup where phenotype–genotype correlations have been clearly established between the size of the repeat and the age at onset, and in some cases the clinical presentation. Another example is the retinopathy that only occurs in SCA7 patients with medium or large expansion size. This is far less evident for conventional variants although the nature of the variants may play a role in the considerable phenotypic variability in the allelic SCA15/SCA16 and SCA29 diseases due to *ITPR1* deletions versus point mutations, respectively. Their clinical presentations can then extend from non-progressive congenital cerebellar ataxia with delayed motor development in SCA29 or in Gillespie syndrome when associating visual impairment and hypotonia, to adult onset slowly progressive ataxia with hyperreflexia (SCA15) (MIM#147265).

A better understanding of the different phenotypic presentations according to the variants may lead to better follow-up for patients regarding the onset of specific signs such as vision loss or dementia. Interestingly, even if conventional mutations only represent a few percent of cases (Coutelier et al. 2017), a comparison of the clinical profiles of patients with conventional mutations or CAG repeat expansions showed that the evolution is significantly slower and less severe in the former (Monin et al. 2015). This information sometimes helps to guide the genetic diagnosis, prioritizing or not the quest for repeat expansions at first.

5 Biomarkers and Treatment

The developments in genetic diagnostic approaches have enhanced the diagnosis of ADCAs and related genetic diseases (Yahia and Stevanin 2021). However, these improvements in ADCA diagnosis have far outpaced the development of therapies for these conditions and the identification of biomarkers to assess their severity and response to treatments. As with most neurogenetic conditions, there is no cure for cerebellar degeneration and its symptoms, or for the detrimental effect on patient's quality of life. However, the battery of promising pharmacological, non-pharmacological, and novel gene therapy interventions to manage ADCA is expanding. Generally, pharmacological and non-pharmacological interventions are symptom-oriented such as L-Dopa for responsive Parkinsonism, or preserving motor coordination with exercises and exergames, respectively (Ilg et al. 2012; Ayvat et al. 2022). On the other end, gene therapy-based interventions aim to modify the disease etiology itself.

Overall, EAs cause less disability compared to SCAs and have more therapeutic options. Most therapeutic interventions in EAs are phenotype-driven rather than genotype-driven (Silveira-Moriyama et al. 2018). However, some exceptions exist; for example, fampridine, a potassium channel blocker, and acetazolamide are

effective in reducing the number of attacks in EA2 and some other EAs (Muth et al. 2021). Regarding SCAs, there is no disease-modifying treatment yet; however, several promising medications are at different stages of clinical trials (Brooker et al. 2021). The interest on riluzole, a potent neuroprotective molecule through its modulation of excitatory transmission in SCAs and Friedreich ataxia patients (Romano et al. 2015), is debated in light of recent results raising doubts about its efficacy in SCA2 patients (Coarelli et al. 2022).

Antisense oligonucleotides (ASOs) are a promising therapeutic approach that has been tested in ADCA animal and cell models (Hauser et al. 2021; Zhang et al. 2021). ASOs are short synthetic oligonucleotides designed to target specific RNAs to modulate their functions through multiple mechanisms depending on their chemistry, sequence, and target (Silva et al. 2020). These mechanisms include inhibiting protein synthesis machinery, modifying splicing, interfering with RNA capping and tailing, and interfering with microRNA-directed RNA regulation (Silva et al. 2020). Most strategies are targeting specific exonic sequences outside the repeat, but with consequences possibly on the expression of the normal allele except when targeting allele specific polymorphisms in cis of the expansion. Other strategies are targeting the repeat itself. The effect of these strategies on the toxic species produced by RAN translation has not been explored yet, however. Interestingly, clinical trials to evaluate ASO for treating Huntington's disease, another repeat expansion disorder, have started with promising initial data (Tabrizi et al. 2019; Arnold 2021; Ghanekar et al. 2022) but with very disappointing results at present (Kingwell 2021). A second promising gene therapy-based approach is correcting pathogenic genetic alterations through viral vectors. There are multiple viral vectors and the choice depends on the disease and the modality of cell targeting, in vivo versus ex vivo (Li and Samulski 2020). Adeno-associated virus vector has been approved for treating neurological and eye diseases (Li and Samulski 2020), thus promising for treating ADCA. Another promising approach for ADCA treatment is the clustered regularly interspersed short palindromic repeats (CRISPR)-Cas9 directed gene editing technique. This technique has successfully corrected disease-causing mutations in several models of polyglutamine diseases (Karwacka and Olejniczak 2022), including patients-derived fibroblasts (He et al. 2021), and animal models (Yang et al. 2017). However, further studies are required to establish the safety and efficacy of CRISPR-Cas9 gene editing in treating ADCA and overcome its limitations, particularly the off-target issue.

An interesting therapeutic intervention that we also want to highlight is the substitution of deficient substances and metabolites for treating ADCA. An example is the administration of oral DHA for treating SCA38 (Manes et al. 2017, 2019). SCA38 is caused by mutations in *ELOVL5* encoding an enzyme involved in the synthesis of polyunsaturated fatty acids, including docosahexaenoic acid (Di Gregorio et al. 2014). Also, DHA is used as a biomarker for the disease (Di Gregorio et al. 2014).

Indeed, the lack of adequate reliable biomarkers for most forms is still a major hurdle to treating numerous SCAs. There are only a few non-specific predictors for many SCAs, including clinical, serological, digital, radiological, and physiological

predictors (Klockgether et al. 2019; Kim et al. 2021). Age, CAG repeat length, and the associated clinical features, e.g., double vision in SCA3, are important classical predictors for individuals at risk of SCA to develop ataxia (Jacobi et al. 2020). Electrophysiological predictors were suggested for some SCA subtypes, e.g., multifocal electroretinogram for SCA1 and F-wave in SCA3 (Cai et al. 2020; Ziccardi et al. 2021) as well as MRI-based biomarkers (Joers et al. 2018; Jacobi et al. 2020). Serum neurofilament light chain is a severity predictor for several SCAs and correlates with their degree of disability (Coarelli et al. 2021; Shin et al. 2021). Interestingly, a recent study detected serum neurofilament light chain changes in the pre-ataxic stage of SCA1 subjects and preceded volumetric MRI alterations, suggesting a more prominent role for this biomarker in the future (Wilke et al. 2022). Wearable sensors have been used as digital biomarkers to estimate motor impairment in SCA patients, including those in their pre-SCA prodromal period (Shah et al. 2021; Velázquez-Pérez et al. 2021).

6 Conclusions

The genetic and clinical heterogeneity of ADCA is a real challenge in diagnosis. Even if NGS and long-read sequencing are now reducing diagnostic wandering, the high frequency of undiagnosed patients, either because no variant was found or because the variants identified were inconclusive, is still a real issue in daily practice. This calls for international collaborative sharing of information on variants and for the use of the latest diagnostic strategies, such as the SOLVE-RD (https://solve-rd.eu/) and ATAXIA-Global (https://ataxia-global-initiative.net/) initiatives.

Since ADCA is quite diverse in clinical and genetic origin, the identification of common therapeutic strategies for multiple entities is challenging. There is a cross-talk between the mechanisms involved that hinders a discrete delineation between the involved pathological mechanisms (Klockgether et al. 2019). For example, EAs and SCAs are not restricted to one category of gene function. In addition, other mechanisms are indirectly implicated in ADCAs' pathogenesis, sometimes as secondary events, e.g., abnormal neuronal development, abnormal signal transduction, and others, further complicating such a delineation (Sullivan et al. 2019; Malik et al. 2021). In polyglutamine SCAs, multiple cell pathways are affected concomitantly. Most hope has then been placed on the development of RNA targeting-based strategies in polyglutamine SCAs, because of the convincing results in animal models and the fact that these strategies can target the disease process very early. It is likely that pharmacological compounds will also be identified in the future, in particular to target signal transduction and channel ADCAs. Alternative strategies using molecules targeting translation to help truncating variants escape the non-sense-mediated mRNA decay may potentially benefit some patients, but the delivery of these molecules to the brain and the absence of toxicity are issues that will need to be solved.

Other challenges exist, including the low prevalence of these conditions and their phenotypic and genetic heterogeneity (Brooker et al. 2021). The variability of phenotypic presentation is largely understudied. Multicentric collaborations and animal models may be beneficial for overcoming some of these obstacles (Lin et al. 2020; Cendelin et al. 2021).

Acknowledgments We are grateful to Alexis Brice and Nick Barton for critical reading of the manuscript.

Funding Information The authors' work is financially supported by ATAXIA-UK (to GS), the European Union's Horizon 2020 research and innovation program under grant agreement No. 779257 (to GS), and the association Connaître les Syndromes Cérébelleux (to GS).

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Autosomal and X-Linked Degenerative Ataxias: From Genetics to Promising Therapeutics

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Abstract Autosomal recessive cerebellar ataxias (ARCAs) refer to a large group of neurodegenerative disorders mainly affecting the cerebellum and the nervous system. ARCAs are characterized by important genetic heterogeneity and complex phenotypes. Because of their rarity and heterogeneity, it is challenging to rapidly advance our understanding in addition to discovering viable symptomatic and, most importantly, disease-modifying treatments. Significant advances have been recently achieved regarding the genetic basis of autosomal recessive and X-linked cerebellar ataxias. Unfortunately, the pathophysiology of most ARCAs is poorly character-

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_5

ized. For most ARCAs, clinical management consists in supplying symptomatic treatments. However, many new therapeutic strategies have emerged. They range from reducing the debilitating effects of ARCAs to exploring curative strategies. The aim of this chapter is to discuss fundamental and novel genetic aspects of ARCAs and X-linked cerebellar ataxias, focusing specifically on the fragile X tremor ataxia syndrome (FXTAS). We summarize clinical features, pathophysiology, diagnosis, currently available therapies, and novel research for the most frequent ARCAs. We also present examples of how novel and cutting-edge therapeutic tools including the clustered regularly interspaced short palindromic repeats (CRISPR) approach, antisense oligonucleotides (ASOs), and stem cells may lead to disease-modifying and ultimately curative treatment for ARCAs. The emphasis is made on new and ongoing research for the most frequent ARCAs. We will discuss promising future therapeutic strategies as well.

Keywords Recessive ataxia · FXTAS · Friedreich's ataxia · Ataxia with oculomotor apraxia · Ataxia with vitamin E deficiency · Ataxia-telangiectasia · ARSACS

1 Introduction

Autosomal recessive cerebellar ataxias (ARCAs) and X-linked cerebellar ataxias form a large group of neurodegenerative disorders affecting the cerebellum and the nervous system. Recessive disorders are inherited when an individual has two copies of a mutated gene, including homozygous mutations as well as compound heterozygous mutations. X-linked disorders are the result of mutated genes located on the X chromosome and can be either recessive or semidominant (Zanni and Bertini 2018). ARCAs now consist of more than 40 distinct clinical entities and an even greater number of genes associated with these disorders (Embirucu et al. 2009). Most patients present with highly variable phenotypes, which are leading to an important clinical heterogeneity, even when bearing the same mutations. One of the most prevalent ARCAs, namely Friedreich ataxia (FRDA), affects approximately 1/50,000 individuals with the same male/female ratio (Koenig 2003). Other ARCAs, such as autosomal-recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) were reported worldwide but are more frequent in certain regions due to a founder effect (Bouchard et al. 1998). It is possible that other ARCAs are yet to be discovered because of their rareness.

ARCAs are generally early-onset diseases that are commonly diagnosed before the age of 20 (Palau and Espinos 2006). The clinical presentation of ARCAs often consists of slowly progressive neurological symptoms including gait disturbances, poor coordination of eye movements, speech alteration, and reduced hand dexterity (Kwei and Kuo 2020). In some cases, systemic symptoms can be observed, such as heart disease in FRDA (Hanson et al. 2019). Clinicians must generally consider family history, clinical findings, neuroimaging, and molecular diagnosis to establish the

Table 1 Currently ongoing clinical trials on autosomal and X-linked degenerative ataxias

Diseases	Clinicaltrials.gov study identifier	Treatment or drug	Clinical trial phase
Friedreich ataxia	NCT03933163 NCT04801303 NCT04577352 NCT04102501 NCT02255435	Resveratrol Calcitriol Vatiquinone RT001 (11,11-di-deutero-linoleic acid ethyl ester) Omaveloxolone	Phase 2 Phase 4 Phases 2 and 3 Phase 3 Phase 2
Ataxia telangiectasia	NCT04870866 NCT03309150	Nicotinamide ribonucleoside M6620 (inhibitor of ataxia telangiectasia and Rad3 related (ATR) kinase) monotherapy or in combination with carboplatin or paclitaxel	Phase 2 Phase 1

diagnosis because of the heterogeneity of ARCAs (Bird 1993). The diversity and complexity of ARCAs are also a challenge to their treatment. Hopefully, in recent years, advances in molecular gene edition, like the clustered regularly interspaced short palindromic repeats (CRISPR) technique, have given great hope to find new gene therapies that will eventually prevent the devastating effects of cerebellar ataxias.

In this chapter, we will provide an overview of clinical features, pathophysiology, and diagnostic criteria for ARCAs. These include FRDA, ARSACS, synaptic nuclear envelope protein 1 (SYNE1)-related ataxia, and ataxia telangiectasia (AT). Less frequent ataxias will also be considered, including ataxia with oculomotor apraxia type 1 (AOA1) and type 2 (AOA2), spastic paraplegia type 7 (SPG7), ataxia with vitamin E deficiency (AVED), and FXTAS, which is included in the X-linked progressive (or degenerative) ataxias group of disorders. Promising therapeutic strategies will also be presented. Emphasis will be placed on clinical therapies, as well as on currently ongoing clinical trials (Table 1) and new research on future therapeutic options for the most frequent ARCAs as inspired by cutting-edge approaches with dominant forms of ataxias.

2 Classification

Establishing a classification of ARCAs is challenging because of its complex phenotype and high genetic heterogeneity. Up to recently, there was a serious need for a consensual classification of ARCAs that would facilitate clinical diagnosis. Two classifications were recently proposed to the scientific community. The first classification was led by the Society for Research on the Cerebellum Ataxias and involved a Task Force of 11 neurologists (Beaudin et al. 2017, 2019). This classification was established based on clinical symptoms and pathophysiological features, providing a unified understanding of autosomal recessive cerebellar disorders for clinicians and researchers. It includes the classification of 59 disorders (Beaudin et al. 2019). The second one was proposed by the International Parkinson and Movement Disorder Society Task Force and their classification includes 62 disorders with

ataxia as the predominant feature (Rossi et al. 2018). It proposes a new nomenclature for the genetically confirmed ARCAs to guide the molecular diagnostic testing and to facilitate its interpretation.

3 Friedreich Ataxia (FRDA)

3.1 Clinical Features

Friedreich's ataxia (MIM#229300) is the most common inherited ataxia (Koenig 2003). The gradient prevalence of FRDA in Europe colocalizes with the chromosomal R1b marker discovered in the same region. Two main reasons could explain this gradient: Paleolithic migrations out of the Franco-Cantabrian Ice Age refuge or Neolithic migrations into west Europe with the spread of agriculture (Vankan 2013) This may be one of the reasons explaining that this disease is uncommon in Sub-Saharan African groups (Labuda et al. 2000) and mostly affects Caucasian groups. The average age of disease onset is 15 years old but, cases of late-onset and very-late-onset have been observed in individuals between 25 and 40 years old (Cook and Giunti 2017). There is an important diversity of clinical phenotypes. FRDA always involves gait and limb ataxia, dysarthria, and loss of lower limb reflexes (Cook and Giunti 2017; Koeppen 2011). There are also systemic effects related to this disease such as cardiomyopathy, diabetes, and skeletal abnormalities (Holt et al. 2019). Children suffering of FRDA may appear clumsy, and present gross and fine motor difficulties in comparison to nonaffected siblings. Ataxia may be preceded by scoliosis and pes cavus (Koeppen 2011). Also, cardiomyopathy might be one of the first clinical manifestations in some patients, while diabetes mellitus is always a late complication (Koeppen 2011). The most common cause of death in FRDA is cardiac dysfunction, namely, congestive heart failure or arrhythmia, and the average age at death was reported as 36.5 years old (range of 12–87 years old) in a large retrospective study (Cook and Giunti 2017; Tsou et al. 2011).

3.2 Pathophysiology

FRDA is caused by genetic alteration to the *FXN* gene, which encodes a highly conserved 220 amino acid mitochondrial protein called frataxin (Santos et al. 2010). The *FXN* gene span of 95 kb of the genomic sequence contained in 7 exons is located on the long arm of chromosome 9 (9q13-21.1) and is characterized by a homozygous expansion of guanine-adenine-adenine (GAA) trinucleotide in the first intronic region. People affected by this disease can present up to 1300 GAA repeats, with an average of 400 repeats, while healthy individuals generally have fewer than 36 trinucleotide repetitions. However, about 2–4% of patients have heterozygous mutations, which result in atypical phenotypes (Cossee et al. 1999).

Repeat expansions are inherently unstable (dynamic) and share numerous genetic features, they: (1) arise from normally existing polymorphic repeats; (2) often change size when transmitted to next generations; (3) tend to cause more severe and earlier onset disease when longer (Delatycki and Bidichandani 2019; Filla et al. 1996); and (4) present variable phenotype, primarily reflecting differences in repeat size. Homozygous patients for the expansion have marked reduced levels of frataxin (Li et al. 2016). A small fraction of FRDA patients are compound heterozygotes, that is, bearing a single GAA expansion on one allele coupled with a deletion or loss-of-function mutation on the second allele (Cossee et al. 1999).

Frataxin is a 14.2 kDa mitochondrial protein involved in the regulation of iron transport and the biosynthesis of the iron-sulfur cluster (ISC). In fact, iron homeostasis disruption is also associated with deficiency in proteins containing ISC cofactors like mitochondrial respiratory complexes, Krebs cycle proteins, DNA repair, and replication proteins (Holt et al. 2019). Frataxin is mostly expressed in the dorsal root ganglia (DRG), spinal cord, cerebellar dentate nuclei, cerebral cortex, pancreas, heart, liver, and skeletal muscle (Cossee et al. 1997), which correspond to the main pathological sites associated with FRDA. Most of the neurological manifestations originate from the DRG, dentate nuclei of the cerebellum, posterior columns, spinocerebellar and corticospinal tracts of the spinal cord and peripheral nerves (Koeppen 2011). In addition, lymphoblasts *FXN* transcripts and protein levels were found to be 5–30% higher in affected individuals compared to healthy people (Chutake et al. 2014).

3.3 Diagnosis and Treatment

The diagnosis is generally established by clinical examination, and it is confirmed by molecular genetic testing. A large fiber sensory axonal neuropathy can be revealed in nerve conduction investigations. However, the absence of neuropathy does not exclude the diagnosis (Collins 2013). Early diagnosis and treatment are important for neurodegenerative disorders like FRDA and it explains why we need to identify specific biomarkers. A study showed that epigenetic modifications, particularly miRNA-based regulatory mechanisms, might be linked to FRDA (Viswambharan et al. 2017). This function is currently being investigated. In fact, Frataxin expression was found to be affected by genetic variations producing miRNA target sites in the 3′-UTR of *FXN* (Viswambharan et al. 2017). To date, there is no cure for FRDA and symptomatic treatments may include multidisciplinary considerations because FRDA is a multisystem disorder (Lynch et al. 2021a). As musculoskeletal complications are common, physiotherapy is often required as well as surgery to treat scoliosis. Various clinical scales are used to evaluate the progression of the disease, such as the Friedreich's Ataxia Rating Scale, the International Cooperative Ataxia Rating Scale, and the Scale for the Assessment and Rating of Ataxia.

3.4 Current Clinical Research

At least two therapeutic avenues have been investigated with more relative success (Cook and Giunti 2017). The first strategy focuses on the management of the cellular oxidative stress caused by the mitochondrial dysfunction. In FRDA, oxidative stress was suggested to be undermined by the inability to efficiently activate the NF-E2 p45-related factor 2 (Nrf2) pathway (Paupe et al. 2009). Defects in the Nrf2 pathway have been described in several in vitro and in vivo models of FRDA and have been associated with the mitochondrial impairment and the oxidative imbalance (Paupe et al. 2009; Lynch and Farmer 2021; D'Oria et al. 2013). Many antioxidants are being investigated as a treatment for FRDA.

Of particular interest, the benzoquinone idebenone is an antioxidant that has been widely explored for its potential to alleviate symptoms associated with frataxin deficiency and it has been shown to be safe and well tolerated in humans (Lagedrost et al. 2011; Lynch et al. 2010; Meier et al. 2012). Idebenone medication enhances echocardiographic parameters in patients with FRDA, such as hypertrophy (Ribai et al. 2007). However, conflicting results exist regarding its potential benefits regarding cardiomyopathy (Giovanni et al. 2015). Indeed, randomized double-blind placebo-controlled trials have failed to establish any robust evidence of benefit for neurological or cardiac function (Lagedrost et al. 2011; Lynch et al. 2010; Meier et al. 2012). A clinical pilot study to determine the efficacy, safety, and tolerability of triple therapy with deferiprone, idebenone, and riboflavin in FRDA patients has also concluded that there is an uncertain benefit on the neurological and heart functions of this triple therapy in FRDA, as measured by changes in the scale for the assessment and rating of ataxia (SARA) and echocardiography parameters (Arpa et al. 2014).

Omaveloxolone, an Nrf2 activator combined with a nuclear factor kappa B (NF-κB) suppressor that prevents the ubiquitination of Nrf2 and thus decreases its turnover, was shown to improve neurological function (Lynch et al. 2021b) and is currently in clinical trial phase 2 (NCT02255435). Another phase 2 clinical trials are currently underway, involving resveratrol (NCT03933163), also studied in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). An improvement in neurological function in FRDA patients with high-dose administration was reported (Yiu et al. 2015). A Phase 3 clinical trial on Vatiquinone, a drug targeting an oxidoreductase involved in the synthesis of an essential compound playing a role in the control of oxidative stress is also underway (NCT04577352). The previous Phase 2 clinical trial (NCT01962363) resulted in an improved neurological function and disease progression (Zesiewicz et al. 2018a). Also, a (Phase 3) clinical trial on RT001, a lipid peroxidation inhibitor is underway (NCT0410250). RT001 is a deuterated ethyl linoleate. It showed significant improvements in maximal exercise workload, giving patients hope to overcome severe fatigue during task performance (Zesiewicz et al. 2018b). Finally, Britti and collaborators reported that calcitriol, an active form of vitamin D physiologically synthesized by mitochondria, increased the level of mature frataxin in DRG neurons, frataxin-deficient cardiomyocytes, and

in lymphoblastoid cell lines derived from FRDA patients, leading to improved mitochondrial function and cell survival (Britti et al. 2021). This promising approach is currently being studied (Phase 4 clinical trial) (NCT04801303).

The second strategy focuses on re-establishing the iron homeostasis. Indeed, iron accumulation is a hallmark feature of FRDA and, initially, iron accumulation was suggested to be a primary pathogenic event triggered by *FXN* deficiency (reviewed in Llorens et al. (2019)). In cellular models, the iron chelator deferiprone has been shown to pass through the blood–brain barrier and to promote the elimination of intracellular iron (Pandolfo and Hausmann 2013; Boddaert et al. 2007). Despite encouraging safety results (Elincx-Benizri et al. 2016) and documented benefit to cardiac health of FRDA patients (Pandolfo and Hausmann 2013), choosing the optimal dosage is very challenging (Crisponi et al. 2015). In addition, depletion of mitochondrial iron was demonstrated to induce mitophagy, which may increase the risk of adverse effect (Hara et al. 2020).

4 Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)

4.1 Clinical Features

ARSACS (MIM#270770) was first described in the Charlevoix and Saguenay-Lac-St-Jean regions (astern Canada), where it is one of the most common inherited ataxias due to a founder effect. Cases of ARSACS were reported in more than 20 countries leading to a varied clinical spectrum of the disease (Dupre et al. 2006). Approximately 80% of ARSACS patients present the classic symptom triad of cerebellar, pyramidal, and neuropathic involvement before the age of 30 (Bouchard et al. 1998; Synofzik et al. 2013). First signs of the disease are often the result of pyramidal damage and generally first occur in childhood, appearing as imbalances, falls, deformities of the feet and hands, or spasticity in the lower limbs (Bouchard et al. 1998; Bereznyakova and Dupre 2018; Dupre et al. 2008). With time, symptoms become more present, and cerebellar damage will lead to speech, writing, or learning difficulties (Dupre et al. 2008; Duquette et al. 2013). During adolescence, ataxia-associated symptoms are more pronounced and lead to difficulties in the execution of fine movements or impaired coordination, which is explained by increased tendon reflexes (Dupre et al. 2008; Duquette et al. 2013). Neuropathic damages can also be detected by electromyography (EMG), which generally shows obvious signs of denervation and demyelination in distal muscles by the 20s (Bouhlal et al. 2011). This disease feature was confirmed in nerve biopsies (Peyronnard et al. 1979; Takiyama 2006). Signs of distal amyotrophy can be severe, proprioceptive sensation in the lower limbs decreases, and Achilles reflexes decline or completely disappear with age (Bouchard et al. 1998; Bereznyakova and Dupre 2018). In addition, peripheral involvement usually increases after the age of 30. By

the age of 40, affected patients often rely on a wheelchair (Bereznyakova and Dupre 2018). Magnetic resonance imaging (MRI) studies have revealed atrophy of the vermis, cerebellar atrophy, and loss of myelin in the corticospinal and posterior spinocerebellar tracts (Dupre et al. 2006, 2008; Bouhlal et al. 2011). Besides these progressive symptoms, other nonprogressive manifestations were observed, such as saccadic eye tracking or retinal nerve fiber hypermyelination (Bouchard et al. 1998; Garcia-Martin et al. 2013). The average life expectancy of ARSACS patients is estimated to be in the range of 51–61 years old (Bouchard et al. 1998; Garcia-Martin et al. 2013).

4.2 Pathophysiology

The sacsin molecular chaperone (*SACS*) gene is mapped on the long arm of chromosome 13 (13q12.12) and contains a total of 10 exons. Among them, 9 exons are coding exons including a gigantic 12,794 pb exon. *SACS* encode the protein sacsin (Ouyang et al. 2006; Engert et al. 2000). Sacsin is a 4579 amino acid protein highly expressed in Purkinje cells, precerebellar nuclei, and corticospinal motor neurons (Ouyang et al. 2006; Engert et al. 2000; Parfitt et al. 2009). The protein is also found in fibroblasts, skeletal muscle, and, at lower levels, in the pancreas (Engert et al. 2000). Its role is not fully understood but it was shown to be involved in protein degradation, proper protein conformation (through heat shock protein homology domain), ATP hydrolysis, organization of the intermediate filaments network, and mitochondrial fission processes (Engert et al. 2000; Parfitt et al. 2009; Romano et al. 2013; Girard et al. 2012; Anderson et al. 2010). Causal mutations in the *SACS* gene were suggested to create a loss of function (Engert et al. 2000; Kozlov et al. 2011). In the Charlevoix and Saguenay-Lac-St-Jean regions, most cases display both or one of the two following mutations in the SACS gene: a deletion producing a frameshift mutation (94% of carrier chromosomes), and a nonsense mutation expected to introduce a premature stop codon (3%) (Engert et al. 2000).

Studies using animal models, primary cell lines, and fibroblasts derived from ARSACS patients have shown that there is a significant decrease in the level of sacsin (Girard et al. 2012; Lariviere et al. 2019; Bradshaw et al. 2016). Pathogenic sacsin alterations lead to a disruption of mitochondrial homeostasis with hyperfused mitochondria so-called balloon-like accumulating in neuron cell bodies (Girard et al. 2012; Bradshaw et al. 2016) as well as increased mitophagy and formation of reactive oxygen species (ROS) (Morani et al. 2019; Duncan et al. 2017). It also leads to the formation of perinuclear neurofilament bundles (Lariviere et al. 2015, 2019; Duncan et al. 2017) as well as Purkinje cell degeneration (Girard et al. 2012; Lariviere et al. 2015, 2019).

4.3 Diagnosis and Treatment

Currently, diagnosis of the disease includes neurological examinations, MRI, nerve and motor conduction studies, and retinal examination (Bouhlal et al. 2011). Molecular genetic testing is also available for establishing the ARSACS diagnosis (Vermeer et al. 2009). Moreover, a disease severity index has been established. The index includes eight tests that evaluate the lower and upper limbs as well as mobility, and was designed to consider cerebellar, pyramidal, and neuropathic disorders (Gagnon et al. 2019). While ARSACS remains an incurable disorder, patients can receive special cares, such as occupational, speech, and physical therapies. Patients are often treated with baclofen to control spasticity and anticholinergics may be given to relieve urinary problems (Bereznyakova and Dupre 2018).

4.4 Current Clinical Research

Docosahexaenoic acid (DHA), known for its neuroprotective properties and for its ability to stimulate autophagy, has successfully been used in patients with spinocerebellar ataxia 38 (Manes et al. 2017, 2019). Prior investigations had been conducted in an FRDA mouse model (Abeti et al. 2015). Interestingly, regular intake of DHA for a 20-month period appeared to stabilize clinical symptoms in two siblings affected with ARSACS (Ricca et al. 2020).

From a more fundamental perspective, treating dermal fibroblasts derived from ARSACS patients with Idebenone, an analog of coenzyme Q10 (CoQ10) and previously studied in FRDA, showed a significant decrease in ROS-positive cells (Martinelli et al. 2020; Parkinson et al. 2013). More recently, the same research group has focused on the benefits of Resveratrol in vitro, a known drug for its antioxidant properties and for its positive effects on neurodegenerative diseases such as Alzheimer's disease (AD) (Loureiro et al. 2017). Another team also demonstrated that Resveratrol treatments contributed to a reduction in ROS levels in ARSACS-fibroblasts (Şen et al. 2021). Inhibition of Hsp90 is another track of investigation. Nethisinghe and collaborators have tested the therapeutic potential of Hsp90 inhibition with the molecule KU-32, a C-terminal-domain-targeted compound (Nethisinghe et al. 2021). The compound was found to reduce vimentin bundling in carrier and patient cells and to restore the mitochondrial electron transport chain.

Recently, a proteomic study compared the cell lysates of fibroblasts isolated from ARSACS patients and SH-SY5Y neuroblastoma cell line invalidated in sacsin with healthy control cells. Data analysis revealed deregulation in neuroinflammation, synaptogenesis, and cell engulfment in the ARSACS and sacsin-deficient SH-SY5Y cell populations compared to the control, leading to other potential therapeutic targets (Morani et al. 2020).

5 SYNE-1-Related Ataxia (ARCA1 – SCAR8)

5.1 Clinical Features

This slowly progressive ataxia was first described in the province of Quebec in Canada (Dupre et al. 2007). In 2007, Gros-Louis and colleagues described an unusual type of inherited cerebellar ataxia called autosomal recessive cerebellar ataxia type 1 (ARCA1) (MIM#610743). It was also called spinocerebellar ataxia autosomal recessive 8 (SCAR8) and more recently, SYNE-1-related ataxia in reference to the affected protein. The median age of onset in the French-Canadian SYNE1 region is 31 years old (most cases within 17–50 years old). The onset of the disease generally occurs in adolescence to early adulthood in other groups (median 17 years old; most cases within 6–42 years old) (Mademan et al. 2016). Cerebellar and extracerebellar symptoms are commonly found in patients (Mademan et al. 2016). Dysarthria, cerebellar ataxia, or both phenotypes can occur simultaneously. Following the start of the conditions, individuals acquire dysmetria, brisk lower extremity tendon reflexes, and mild abnormalities in saccades and smooth pursuit. Extrapyramidal symptoms, retinopathy, cardiomyopathy, sensory abnormalities, or autonomic disturbances are not seen in ARCA-1 individuals (Beaudin et al. 1993). They may also display cognitive deficits without psychiatric comorbidities (Laforce Jr. et al. 2010; Valentina Castillo et al. 2021).

5.2 Pathophysiology

Pathogenic mutations in the *SYNE1* gene encoding the synaptic nuclear envelope protein 1 (SYNE-1), also known as nesprin 1, are the cause of this disease. Located on the short arm of chromosome 6 (6p5), SYNE1 is one of the biggest genes in the human genome. It is 147 exons wide and it is transcribed into a 27,652-bp mRNA, which translates in an 8797 amino acid protein (Zhang et al. 2002). Four truncating mutations were initially discovered to cause SYNE-1 ataxia as well as two splice site mutations and one deletion. Compound heterozygous mutations were also identified in the *SYNE1* gene (Gros-Louis et al. 2007). The result of those seven loss-of-function mutations is expected to be protein truncation (Noreau et al. 2013; Duan et al. 2021). SYNE-1 is expressed in Purkinje cells, olivary bodies, and in myocites (Arias et al. 2022). One of its functions is to form a link between the actin cytoskeleton and the organelles (Baumann et al. 2017). Dysfunction of the protein contributes to disrupting signaling between neurons in the cerebellum. Affected individuals may also suffer cognitive impairment (Laforce Jr. et al. 2010).

5.3 Diagnosis and Treatment

Diagnosis is generally made through MRI findings, which show diffuse cerebellar atrophy without brainstem involvement after only a few years of evolution (Dupre et al. 2007) and using molecular genetic testing for the presence of SYNE-1 biallelic pathogenic variants (Beaudin et al. 1993). In most cases, nerve conduction studies come back negative, but neuropathies can occasionally be detected (Synofzik et al. 2016). MRI scans may appear normal more but may also show acute or chronic neurogenic changes in people with clinical evidence of motor neuron dysfunction (Izumi et al. 2013). There is no curative treatment for this disease. Clinical management's efforts aim at improving patients' mobility while reducing the risk of side effects. A multidisciplinary team should personalize follow-up of patients (Beaudin et al. 1993).

5.4 Current Clinical Research

To our knowledge, few therapeutics are in development for SYNE-1-related ataxia. One of them is the CAD-1883 drug, a small conductance calcium-sensitive potassium channel positive allosteric modulator. A phase 1 clinical study has been already done and a phase 2 trial has been planned to confirm the safety and tolerability but is currently on hold (NCT04301284). Despite a very limited therapeutic perspective, fundamental progress to understand the exact function of nesprin is being made in other tissues. In fact, nesprin is known to be pathologically involved in Emery-Dreifuss muscular dystrophy (EDMD) type 4, a disease caused by disruptions of the nesprin/lamin/emerin interactions in cardiac and skeletal muscle cells (Holt et al. 2019; Janin and Gache 2018; Madej-Pilarczyk 2018; Fanin et al. 2015).

6 Ataxia Telangiectasia (AT)

6.1 Clinical Features

Also known as Louis-Bar syndrome, AT (MIM#208900) is a rare genetic form of early-onset autosomal recessive ataxia. The prevalence of AT is estimated to be 1:40,000–1:300,000, with *ATM* allele heterozygosity representing 1.4–2% of the general population (Amirifar et al. 2020). The clinical picture consists of a combination of neurological and systemic symptoms. In particular, AT is characterized by cerebellar atrophy with progressive ataxia, oculocutaneous telangiectasias, oculomotor apraxia, a higher incidence of malignancy (particularly lymphoid malignancy), radiosensitivity, immune deficiency, recurrent sinopulmonary infections, and high levels of alpha-fetoprotein (AFP) in serum (van Os et al. 2016). Generally,

the disease first manifests during early childhood when the toddler is beginning to sit and walk (Rothblum-Oviatt et al. 2016). Affected children are more likely to develop cancer, leukemia and lymphoma being the most frequent forms that were reported. Furthermore, growth retardation is frequently observed in patients who may also develop type 2 diabetes at puberty (van Os et al. 2016). The phenotypic spectrum of AT is large due to the important variability of clinical presentation. Patients with mild or atypical presentation tend to have an adult onset (Tiet et al. 2020). Patients usually have a poor prognostic with an estimated survival time of 25 years. Because of progressive respiratory failure and/or malignancies, AT is usually fatal in the second or third decade of life (Boder and Sedgwick 1958). A British meta-analysis concluded that heterozygous carriers have a shorter life expectancy and a higher risk of cancer, particularly breast cancer and probably digestive tract tumors (van Os et al. 2016).

6.2 Pathophysiology

This disease is caused by a mutation in the ataxia telangiectasia mutated (*ATM*) gene. More than 500 different mutations have been reported to cause this disease (Becker-Catania and Gatti 2001). Splicing, nonsense, and frameshift mutations are the most common *ATM* mutations listed in AT patients (Perlman et al. 2012). The gene is located on the long arm of chromosome 11 (11q22-23), encoding for the ATM protein. It is a serine/threonine protein kinase mediating a variety of cellular functions (Boohaker and Xu 2014). Protein amount and kinase function are two primary factors that determine the age of onset, progression, and clinical symptoms of AT. Absence of kinase activity in both alleles results in more severe phenotypes (Levy and Lang 2018). The gene contains 66 exons spanning approximately 150 (Buzin et al. 2003). More particularly, ATM has a role in DNA double-strand break (DSB) repair by positively influencing the activity of the suppressor protein p53 after DNA damage, thus inhibiting cell proliferation. Malformations such as gonadal dysgenesis, which is seen in AT patients, can also be caused by an alteration of the cell cycle control mechanisms. ATM is also necessary for the synthesis of immunoglobulin and the survival of lymphoid (Riboldi et al. 2021).

6.3 Diagnosis and Treatment

Diagnosis of AT can be challenging for clinicians due to the rarity of the disorder and international guidelines. A combination of clinical features, family history, neuroimaging, and laboratory findings is generally required. Interestingly, the early onset of ataxia within the first decade in the classical form and oculomotor apraxia is usually crucial to guide the diagnostic. Two scales were used by Jackson and colleagues to evaluate the neurological development of AT. The AT index (or Crawford

score) collects data on clinical findings of AT but does not differentiate between hyperkinetic and hypokinetic movement disorders (Jackson et al. 2016). The second scale, the A-T Neurological Examination Scale Toolkit is more detailed. It rates more specific information on ataxia like ocular function, hyperkinetic and hypokinetic movement abnormalities, and corresponds well with the total A-T index (Crawford et al. 2000). Telangiectasias are present in almost all patients and, therefore, represent a critical criterion to make the correct diagnosis. However, telangiectasias cases are not always easy to recognize (Perlman et al. 2012). Also, there were cases of atypica (Rothblum-Oviatt et al. 2016). The concomitant presence of tumors may raise the suspicion of AT. Abnormalities that can be detected in laboratories include high and slowly increasing AFP levels after 2 years of age and low serum levels of IgA, IgG, and IgE subclasses. Lymphopenia can also be observed.

6.4 Current Clinical Research

No curative nor disease-modifying therapy is available for AT. Among other avenues of investigation, glucocorticoids such as dexamethasone have been studied with some success initially (Quarantelli et al. 2013; Broccoletti et al. 2011; Chessa et al. 2014). It has been suggested that dexamethasone can increase the synthesis of an alternative *ATM* transcript that retains some of the full-length ATM functions. However, this hypothesis is still controversial (Pozzi et al. 2020). As the oral administration pathway presents a long-term risk for the patients, a method for encapsulating dexamethasone sodium phosphate into autologous erythrocytes has been proposed (Chessa et al. 2014). This strategy is currently used in an international, multicenter, randomized, prospective, double-blind, placebo-controlled, phase 3 study (NCT02770807). Having already proved its utility in an $Atm^{-/-}$ mouse model (Yang et al. 2021), the intake of nicotinamide ribonucleoside, precursor of NAD+, as a food supplement for 4 months in patients led to an improvement of the ataxia scores, which then decreased with the withdrawal of the treatment (Veenhuis et al. 2021). The nicotinamide ribonucleoside is currently in phase 2 clinical trial as a food supplement for 2 years in AT subjects (NCT04870866). A phase 1 clinical trial is also ongoing to study the long-term effect of M6620 as monotherapy or in combination with carboplatin or paclitaxel (NCT03309150). M6620, an inhibitor of ataxia telangiectasia and Rad3-related (ATR) kinase, has already been used as a monotherapy or in combination with carboplatin, and has shown to have good tolerability and promising effects in patients with advanced cancers (Yap et al. 2020).

In a more fundamental perspective, recent drug discovery strategies have explored the use of induced pluripotent stem cells (iPSCs). Wolvetang and collaborators have investigated the generation of iPSCs from olfactory neurosphere derived of AT patients (Leeson et al. 2021). Such cell population can be used to generate bidimensional and tridimensional (3D) patient-specific neuronal in vitro models enabling fundamental investigations to better understand the underlying neurodegeneration mechanism. Using stem cell-derived brain organoids, inhibition of the

cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway was shown to ameliorate the premature senescence hallmarks of AT-iPSC-derived neurons by preventing neuronal loss and rescuing neuronal function (Aguado et al. 2021). Interestingly, aspirin and small molecule inhibitors can prevent the activation of the cGAS-STING pathway (Aguado et al. 2021). It may represent a promising potential therapeutic target for treating neuropathology in AT patients.

7 Ataxia with Oculomotor Apraxia Type 1 (AOA1)

7.1 Clinical Features

Ataxia with oculomotor apraxia type 1 (MIM#208920; AOA1) is characterized by a slowly progressive cerebellar ataxia, axonal sensorimotor neuropathy, hypoalbuminemia, and hypercholesterolemia with early onset (at an age of 7 in average). Affected individuals then develop areflexia and loss of ambulation leading to quadriplegia from 7 to 10 years after the apparition of the first symptoms (Coutinho et al. 1993). It is followed by oculomotor apraxia (OMA) a few years after onset and progresses into external ophthalmoplegia (Coutinho et al. 1993). Moderate cognitive impairment appears to be a common characteristic of AOA1 (Le Ber et al. 2003). Individuals with OMA cannot normally fix objects in front of them because of horizontal saccades of elevated latency. They also find it difficult to look away; they tend to generally turn their heads before to follow with their eyes. Individuals who have had their heads immobilized were found to be unable to move their eyes (Coutinho et al. 1993). Blinking can also be exaggerated in these individuals. A wheelchair is often required, usually by ages 15–20 years (Coutinho et al. 1993). AOA1 shares similarities with AT. However, people with AOA1 do not present with extra-neurological features, have a later onset, and are less likely to suffer frequent infections.

7.2 Pathophysiology

The *APTX* gene encodes for a protein called aprataxin, a member of the histidine triad superfamily, which plays a role in DNA repair (Meagher and Lightowlers 2014). *APTX* is located in the 9p13 locus and has 9 coding exons (van Minkelen et al. 2015). Aprataxin can modify the broken ends where the DNA damage is located and contribute to the repair through nonhomologous end joining. This protein catalyzes the nucleophilic release of adenylate groups covalently bound to the 5′phosphate terminal region. The same terminal region can then be reused as substrates for DNA ligases (Rass et al. 2007). Because aprataxin is not fully functional for individuals with AOA1, patients have shown enhanced sensibility to a variety of

agents causing single-strand breaks (Garcia-Diaz et al. 2015). To date, more than 20 different mutations in the *APTX* gene are associated with AOA1 (Amouri et al. 2004; Criscuolo et al. 2005; Date et al. 2001; Jacquemont et al. 2003; Moreira et al. 2001a, b; Sekijima et al. 2003; Shimazaki et al. 2002; Tranchant et al. 2003; Castellotti et al. 2011; Rana et al. 2013). A study showed lower levels of CoQ10 in fibroblasts derived from AOA1 patients due to reduced transcription and transduction of prenyl diphosphanate synthass subunit 1 (Garcia-Diaz et al. 2015). However, the factors causing CoQ10 insufficiency induced by *APTX* mutations are still unknown.

7.3 Diagnosis and Treatment

The diagnosis is classically made with an association of clinical findings, which includes family history and genetic analysis (Coutinho et al. 1993). Cerebellar atrophy can be detected by MRI in affected individuals. In addition, the exclusion of AT can be made when oculomotor apraxia is present. Signs of axonal neuropathy can also be described by the EMG in 100% of patients (Coutinho et al. 1993). Some symptoms like hypoalbuminemia and hypercholesterolemia can be treated and prevented with a low-cholesterol diet and lipid-lowering treatment, but patients need regular follow-up with a physician (Coutinho et al. 1993).

7.4 Current Clinical Research

To date, only one clinical trial has been undertaken for AOA1, which is based on CoQ10 deficiency in the muscles of AOA1 patients to assess the benefits of CoQ10 supplementation (NCT02333305) (Le Ber et al. 2007). No result has been reported yet. In recent years, more fundamental research on AOA1 has focused greatly on the physiological function of aprataxin. Aprataxin has been shown to be involved in the repair of DSBs particularly through its ability to remove damaged 3′ and 5′ ends (Ahel et al. 2006; Takahashi et al. 2007). In AOA1, aprataxin lacks this functionality and nonadenylated DNA ligase has been shown to be deficient, resulting in the accumulation of unrepaired DSBs (Takahashi et al. 2007; Reynolds et al. 2009). More recently, Kato and collaborators have shown for the first-time immunological abnormalities in AOA1 patients, such as leukopenia, decreased levels of $CD4^+$ T-lymphocytes, $CD8^+$ T-lymphocytes, and B-lymphocytes, suggesting a new aspect to consider for therapy (Kato et al. 2021). In addition, the disappearance of hyposignal on MRI due to iron deposition in the dentate nuclei has been established as a novel AOA-specific biomarker (Ronsin et al. 2019). Finally, iPSC lines have been established from patient's fibroblasts (Ababneh et al. 2020), which might help to facilitate studies on this disease.

8 Ataxia with Oculomotor Apraxia Type 2 (AOA2)

8.1 Clinical Features

AOA1 and AOA2 share similar clinical findings. A few unique features help differentiate these two types of ataxias, such as serum levels of AFP. Like AOA1, AOA2 (MIM#606002) is a slowly progressive ataxia characterized by cerebellar atrophy, axonal sensorimotor peripheral neuropathy, and an elevated serum level of AFP (Choudry et al. 2018). Other symptoms include occasional oculomotor apraxia in approximately 56% of cases (Al Tassan et al. 2012), strabismus, dystonic postures of the hands, choreic movements, head or postural tremor, extensor plantar responses or sphincter disturbances, and mildly impaired cognitive functions (Le Ber et al. 2004; Mariani et al. 2017). The age of onset varies, with people being affected anywhere between 2 and 30 years old (Mhanni et al. 2016). The disease duration was estimated to range between 2 and 53 years (Moreira and Koenig 1993). Patients with the disease were reported to live close to their 80s (Moreira and Koenig 1993).

8.2 Pathophysiology

AOA2 is caused by a mutation in the *SETX* gene encoding for the senataxin protein, a large C-terminal DNA/RNA helicase of 2677 amino acids (Choudry et al. 2018). Over a hundred homozygous mutations were discovered to date, including nonsense, missense, and splice site variants, as well as small insertions and deletions (Bohlega et al. 2011). Senataxin is expressed in the brain as well as the spinal cord and muscle cells. Of particular interest, senataxin dominant mutations have been linked to a familial form of juvenile amyotrophic lateral sclerosis (Chen et al. 2004). In the last years, it has been shown that the gene involved in AOA2 was induced in an oxygen-dependent manner. Under hypoxic conditions and through the protein kinase R-like endoplasmic reticulum kinase branch of the unfolded protein response, senataxin protected cells from transcription-related DNA damage and apoptosis (Ramachandran et al. 2021). In addition, senataxin is also thought to be involved in autophagy, providing a potential therapeutic target. Indeed, invalidation of the senataxin resulted in an accumulation of ubiquitinated protein, a decrease in the removal of aggregated protein and mitochondrial defects (Richard et al. 2020). This protein is also involved in the DNA damage response. Senataxin was shown to help with the recruitment of Rad51, an essential protein involved in DNA repair (Cohen et al. 2018). It is also thought to be involved in telomere stabilization as well as in cell survival, preventing the translocation of RNA/DNA hybrids that form because of DSBs (Cohen et al. 2018; De Amicis et al. 2011).

8.3 Diagnosis and Treatment

Testing the serum levels of AFP can bring supplemental information for the diagnosis, considering that they can be elevated for AOA2 patients as opposed to AOA1 patients (Dragasevic-Miskovic et al. 2021). However, studies have shown that patients can still be affected by AOA2 without having elevated serum levels of AFP (Paucar et al. 2019). AT should also be considered when oculomotor apraxia and/or high AFP serum concentrations are present. Several AOA2 patients show increased serum creatine kinase concentration (Moreira and Koenig 1993). In addition, young ataxic patients for whom the FRDA and AT diagnostic have been excluded should be tested for the *SETX* gene (Choudry et al. 2018; Mignarri et al. 2015). EMG reveals signs of axonal neuropathy in 90–100% of AOA2 patients (Bohlega et al. 2011). There is no treatment for this disease, but physiotherapy and other occupational therapy can provide some level of relief for patients especially for disabilities caused by peripheral neuropathy. By the age of 30, a wheelchair is usually required. Educational assistance should be provided to compensate for reading and writing difficulties caused by OMA and sensorimotor peripheral neuropathy (Bohlega et al. 2011).

8.4 Current Clinical Research

Most research efforts have focused on elucidating the role of senataxin and its mechanism of action. Report on therapeutic clinical trial or a potential therapeutic avenue is still needed. Thus, in a more fundamental perspective, a model for studying AOA2 has been developed that may help to make further progress by recreating some aspects of the disease as the *SETX* knockout mouse that did not show neurological disorders (Becherel et al. 2013). Indeed, Becherel and colleagues have developed a novel in vitro model using neurons differentiated from patient-derived iPSCs that exhibit a cellular phenotype typical of AOA2 patients, creating a promising tool for studying AOA2 (Becherel et al. 2015).

9 Ataxia with Vitamin E Deficiency (AVED)

9.1 Clinical Features

The first case of ataxia with vitamin E deficiency (AVED) (MIM#227460) was reported by Burck and collaborators in 1981 (Koenig 2003). AVED manifests in late childhood to early teens between the age of 5 and 15 years old (Schuelke 1993). The clinical features are similar to FRDA and include areflexia, loss of proprioception, head dystonia, dysarthria, cognitive decline, and retinitis pigmentosa, which are

also found among other progressive ataxias (Schuelke 1993). Less frequently, glucose intolerance and cardiomyopathy are among the conditions that can present (Mariotti et al. 2004). Cerebellar ataxia is usually the first symptom to appear and gets worse through the years, often leading to a wheelchair-bound state (Hentati et al. 2012). Head dystonia is not always present but is observed in 28–73% of the affected patients (Benomar et al. 2002).

9.2 Pathophysiology

AVED is caused by mutations in the alpha-tocopherol transfer protein (*TTPA*) gene located on the long arm of chromosome 8 (8q13). Recessive mutations in the *TTPA* gene, which include nonsense, missense, and splice-site mutations, as well as minor deletions and insertions, are responsible for the disease. The majority of those who are impacted have private mutations, which are rare genetic mutations usually found only in a single family or a small population (Hentati et al. 2012). His101Gln (H101Q) mutation in the *TTPA* gene appears to be more prevalent than other known mutations (Hoshino et al. 1999). The concentration of vitamin E is then reduced because of the damaged TTPA. Individuals who are homozygous or compound heterozygous with a mutation have nearly total penetrance for AVED. For nonaffected individuals, vitamin E is normally absorbed by the intestine and then secreted into plasma in chylomicron form. During catabolism of chylomicrons, vitamin E is transferred to particles of circulating lipoproteins. The main function of TTPA is to transport RRR-α-tocopherol. This cytosolic liver protein can distinguish between the eighth dietary vitamin E isomers (α-, β-, γ-, δ-tocopherols and α-, β-, γ-, δ-tocotrienols) and preferentially binds RRR-α-tocopherol to very low-density lipoproteins (VLDLs), which are then discharged into the circulation to the tissues (Hentati et al. 2012). When it comes to intestinal absorption of vitamin E and its incorporation into VLDL, individuals with AVED have a normal function (Traber et al. 1990).

9.3 Diagnosis and Treatment

There is no consensus concerning the diagnosis of AVED. However, it is commonly diagnosed when there is an FRDA-like phenotype with a vitamin E deficiency. Indeed, in the absence of intestinal malabsorption, a low blood vitamin E value is used to make the diagnosis (Palau and Espinos 2006). AVED patients usually have <2.5 mg/L and often <1 mg/L of vitamin E serum concentration (normal values 6–15 mg/L) (Koenig 2003). In addition, the lipoprotein profile is expected to be normal, and diseases linked with malabsorption should be ruled out. In most cases, molecular analysis of the *TTPA* gene allows the confirmation of the diagnosis by demonstrating the presence of pathogenic mutations (Hentati et al. 2012). For the

majority of patients, MRI is normal and not specific. However, a study by Mariotti and colleagues showed cerebellar atrophy in approximately half of the reported patients (Mariotti et al. 2004). In the early stage of the disease, the recommended treatment mainly focuses on re-establishing the plasma vitamin E to a high-normal concentration (Schuelke 1993). Moreover, AVED patients need to monitor their plasma vitamin E concentration every 6 months, which is even more crucial in affected children (Gabsi et al. 2001).

9.4 Current Clinical Research

Supplementation in vitamin E was shown to help stop the progression of the neurological symptoms in most cases. Kohlschutter and collaborators have reported an interesting case of a patient with AVED who has been stable for over 36 years on a controlled supplement therapy (Kohlschutter et al. 2020). To our knowledge, there has been no or few research aiming at identifying a new therapeutic strategy for this disease.

10 Spastic Paraplegia Type 7 (SPG7) Ataxia

10.1 Clinical Features

SPG7 was first characterized as a hereditary spastic paraplegia (HSP), whose syndromes are lower extremity weakness and spasticity (Pfeffer et al. 2015; Lagrand and Hageman 2020). However, patients often present with cerebellar ataxia. In fact, it has been shown that 39–57% of all SPG7 patients have clear signs of ataxia (Lagrand and Hageman 2020; van Gassen et al. 2012). Studies also reported that cerebellar ataxia can be the first and most prominent manifestation, particularly for individuals carrying the common SPG7 mutation p.Ala510Val (A510V) (Choquet et al. 2016). In a large cohort of 241 patients that were recruited through the European SPATAX network (https://spatax.wordpress.com/), a spasticity-predominant phenotype of loss-of-function variants and more frequent cerebellar ataxia in late-onset were reported in patients carrying at least 1 A510V variant (Coarelli et al. 2019). Additional symptoms may include spastic dysarthria, dysphagia, pale optic discs, nystagmus, strabismus, ptosis, hearing loss, motor and sensory neuropathy, amyotrophy, scoliosis, pes cavus, and urinary sphincter disturbances (Casari and Marconi 1993). This ataxia usually presents during mid-adult life (Pfeffer et al. 2015). Furthermore, optic nerve atrophy and peripheral neuropathy are common (Lagrand and Hageman 2020; Casari and Marconi 1993).

10.2 Pathophysiology

Recessive mutations in the *SPG7* gene are known to cause SPG7, but this gene is also the cause of other ARCAs (Pfeffer et al. 2015; Haj Salem et al. 2021; Mancini et al. 2019). Rare variants are found on most of the 17 exons of the *SPG7* gene. While the majority of pathogenic *SPG7* variants are missense mutations, other pathogenic DNA variations are also reported such as nonsense, frameshift, and splice site mutations (Casari and Marconi 1993). Paraplegin, the protein encoded by *SPG7*, which has a high frequency of rare mutations of unknown significance, is a member of the protein family AAA (ATPases associated with a variety of cellular activities) (Elleuch et al. 2006). Paraplegin forms the oligomeric m-AAA protease complex with the paralogous AFG3 like matrix AAA peptidase subunit 2 (AFG3L2) protein, which is essential in mitochondrial protein maturation and degradation (Leonhard et al. 2000). The paraplegin-AFG3L2 complex is thus inactivated. Further research is needed to determine how paraplegin alters mitochondrial DNA. More specifically, there is considerable controversy regarding the role of paraplegin in the regulation of mitochondrial permeability transition pores. Nevertheless, this pathophysiological mechanism is of interest for the development of therapeutics. According to Hurst and colleagues, paraplegin can modulate the mitochondrial permeability transition pore through regulation of the basal mitochondrial Ca^{2+} concentration (Hurst et al. 2019) but not all investigations did conclude in the same direction (Klutho et al. 2020).

From a more basic science perspective regarding drug discovery in SPG7 disease, Wali and collaborators have developed a phenotypic assay that will ultimately facilitate high-throughput screening through identification of cell morphology change. They have observed cell morphology's changes following treatment with noscapine, a tubulin-binding drug, in a genotype-dependent manner (Wali et al. 2021). The development of such assay will help in identifying therapeutics in the future. Recent work of this group to further our knowledge on the pathophysiology of SPG7 disease has indicated that the SPG7-derived cell phenotype is characterized as having significantly impaired mitochondrial morphology and functions including reduced oxidative phosphorylation. Indeed, the same team has evaluated mitochondrial function in olfactory neurosphere-derived cells from patients with a variety of *SPG7* mutations that express paraplegin (Wali et al. 2020). SPG7 patient-derived cells had increased paraplegin expression, fragmented mitochondria with low interconnectivity, reduced mitochondrial mass, decreased mitochondrial membrane potential, reduced oxidative phosphorylation, reduced ATP content, increased mitochondrial oxidative stress, and reduced cellular proliferation (Wali et al. 2020).

10.3 Diagnosis and Treatment

Due to the increasing number of genes known to cause HSP, the complexity of gene testing has become more significant (Blackstone et al. 2011). To help the diagnosis, the most common characteristic observed in MRI images is moderate cerebellar

atrophy (Elleuch et al. 2006). Baclofen or tizanidine may help with spasticity and muscle tightness (Casari and Marconi 1993). Half of the SPG7 patients were using a walking aid after an average of 16 years of disability, but the range was from 1 to 70 years (van Gassen et al. 2012). Physical therapy, occupational therapy, and speech therapy assist with daily activities (Casari and Marconi 1993).

10.4 Current Clinical Research

Studies on symptomatic treatment for SPG7 were often described through case reports or through cohorts with very few patients. Though useful, clinical, and genetic heterogeneity inherent to small-size population studies does not allow generalizable conclusions. Examples include studies on transcranial magnetic stimulation (Nardone and Tezzon 2003) and spinal cord stimulation (Ardolino et al. 2021). In a retrospective study, Paparella and colleagues evaluated the efficacy of combining botulinum toxin injection and intensive physical therapy for HSP. The study considered a small number of patients including one with SPG7. The authors report significantly reduced disease severity by the Spastic Paraplegia Rating Scale, reduced muscle tone, and increased walking speed. However, they were unable to untangle the effect of injection from that of intensive physical therapy (Paparella et al. 2020).

In another study, Sambri and colleagues have suggested that paraplegin is required for efficient transient opening of the mitochondrial permeability transition pore that is impaired in both SPG7 patients-derived fibroblasts and primary neurons from *Spg7* knockout mice (Sambri et al. 2020). They have proposed an intriguing pathophysiological mechanism in which dysregulation of mitochondrial permeability transition pore opening at the presynaptic terminal impairs neurotransmitter release leading to ineffective synaptic transmission. In turn, this would increase the expression and activity of sirtuin-3, which promotes deacetylation of cyclophilin D, thus hampering mitochondrial permeability transition pore opening. Interestingly, pharmacological treatment with Bz-423 normalizes synaptic transmission and rescues the motor impairment in the SPG7 mouse model (Sambri et al. 2020).

11 X-Linked Ataxias

11.1 Clinical Features

X-linked cerebellar ataxias are a relatively large group of rare ataxias caused by alterations on the X chromosome. More than 20 genes have been involved up to now (Zanni and Bertini 2018). Main clinical features of this disease consist in hypotonia, development delay, and intellectual disability (Zanni and Bertini 2018). The age of

onset is typically in the first or second decade and the disease can be nonprogressive or slowly progressive. Carrier women are more likely to have no symptoms or fewer manifestations. Most genes involved encode for proteins that are important for normal neuronal processes including synaptic function and brain development. Perhaps one of the most characterized X-linked cerebellar ataxia is the fragile X-associated tremor/ataxia syndrome (FXTAS) (MIM#300623). This specific condition is characterized by an average onset in the 60s for the male carriers of premutations in the fragile X mental retardation 1 (*FMR1*) gene (Leehey et al. 2007). Patients generally display intention tremor and cerebellar ataxia including the development of an ataxic gait in most cases, leading to progressive disability (Jacquemont et al. 2003). As recently reviewed by Cabal-Herrera and collaborators, neuropathy, parkinsonism, and executive dysfunction are commonly associated with FXTAS (Cabal-Herrera et al. 2020; Hall et al. 2016). Other symptoms may include high blood pressure, thyroid disorders, and fibromyalgia (more common in females) (Wheeler et al. 2014). In addition, several manifestations may be observed including dysautonomia, sleep problems, migraine headaches, vestibular dysfunction, olfactory deficit, chronic fatigue, and psychiatric problem (reviewed in Cabal-Herrera et al. (2020)).

11.2 Pathophysiology

FXTAS is caused by an expanded CGG triplet alleles in the premutation range (50–200 repeats) in the *FMR1* gene leading to an elevated production of mRNA, up to eight times more than normal (Hagerman and Hagerman 2016). The *FMR1* gene consists of 17 exons spanning 38 kb of Xq27.3 and it codes for the protein FMRP (fragile X mental retardation protein) (Eichler et al. 1994). Overexpression of the transcript is associated with a toxicity potentially causing the clinical features of the disease. Indeed, the development and the function of neural cells can be affected by the increase in mRNA levels (Zanni and Bertini 2018). Several mechanisms for this have been proposed (Hagerman and Hagerman 2021) in FXTAS, including the sequestration of proteins important for neuronal function such as the RNAse III Drosha (Sellier et al. 2017) and the dysregulation of calcium leading to a mitochondrial dysfunction (Robin et al. 2017). Interestingly, there is a correlation between the extent of the expanded CGG triplet and the severity and the disease (Hagerman and Hagerman 2021). Recently, Dufour and colleagues noted an increased presence of proinflammatory cytokines TNFα and IL-12 in fresh frozen cerebellar tissue from FXTAS cases. Both cytokines were implicated in the pathogenesis of multiple sclerosis, another neurodegenerative disorder that predominantly consists of white matter disease (Dufour et al. 2021).

11.3 Diagnosis and Treatment

Family history is necessary to rule out autosomal or other modes of inheritance (Zanni and Bertini 2018). The National Fragile-X organization has elaborated some criteria to help diagnose FXTAS. They consider that there is a definite presence of the disease when one or two main symptoms are present in any patient who shows neuropathology signs of FXTAS. The National Fragile-X organization also requires for any possible FXTAS patient to have DNA testing for the *FMR1* gene. The CGG repeat size is essential to know since it corresponds with the age of presentation of symptoms and with the severity of motor indications and neuroimaging findings. With the exception of *FMR1*, genetic testing in sporadic males or confirmed X-linked familiar instances should be undertaken only in well-selected patients with a particular clinical and neuroradiologic profile (Zanni and Bertini 2018). Bilateral hyperintensities of the middle cerebellar peduncles on T2-weighted MRI or FLAIR images were found for FXTAS male patients. These radiological findings are discriminating for FXTAS. Brain atrophy is also described but is not a specific finding (Hagerman and Hagerman 2021). There is no effective treatment yet for FXTAS. However, progress in understanding this ataxia has helped developing ways to prevent and improve the neurological manifestations. Because there are many phenotypes for this syndrome, the treatment can vary for each patient (Mila et al. 2018).

11.4 Current Clinical Research

In recent FXTAS clinical trials, benefits regarding cognition and anxiety were reported. Indeed, a follow-up—41 patients—study selected in a cohort of patients initially treated with memantine for 12 months found an improvement in cued memory recall as manifested by an increase in the amplitude in N400 on memantine versus placebo (Yang et al. 2014). In addition, improvement in attention and in self-reported information processing was noted (Yang et al. 2016). Hagerman and colleagues have suggested that medications that can slow cognitive decline in AD such as Donepezil, Rivastigmine, and Galantamine may be useful in FXTAS but have not been subjected to controlled trials (Hagerman and Hagerman 2021). In another trial, allopregnanolone, a neurosteroid, has had some benefits in one patient with alleviation of neuropathy and improvement of ataxia in addition to improvement in cognition (Wang et al. 2017). Finally, in the trial involving ten patients utilizing citicoline (Hall et al. 2020), an over-the-counter endogenous nucleotide and intermediate in the biosynthesis of structural membrane phospholipids, there was stability over time and some mild improvements in attention and in an anxiety measure.

Among other tracks of investigation, there is a deficit in proteins that eliminate extra iron from the cells with the concomitant increased in the deposit of cellular iron (Ariza et al. 2017). Drug-mediated iron relocation, like deferiprone, could

ultimately be helpful to treat iron overload as it has the iron-relocating effect that may alleviate iron accumulation.

12 Preclinical and Future Therapeutic Advances

As of today, there is no approved disease-modifying nor curative treatment for ARCAs and X-linked degenerative ataxias. However, encouraging preclinical studies in the fields of gene therapy and RNA interference are exponentially accumulating and lead the way to identify novel treatments. Disease-modifying therapies are not administered to cure, but to reduce, delay or reverse the progression of life-threatening symptoms, especially when given in the early phase of the disease. It is important to note that all the therapies described below are in preclinical phases and are not yet administered in patients.

12.1 Nucleic Acid-Based Drugs

Targeting the expression of specific ataxia-associated proteins using antisense oligonucleotides (ASOs), and other nucleic acid-based treatments, shows promising in vitro and in vivo data for future human therapeutic applications. ASOs are small-sized and chemically modified RNA-based drugs designed to precisely bind RNA sequences, alter their expression, and then limit the progression of the desired disease (Stephenson and Zamecnik 1978; Dhuri et al. 2020). Over the years, various ASOs candidates have been tested in clinical trials to treat a plethora of incurable diseases, such as cardiovascular, neuromuscular, neurological and inflammatory diseases, of which some have already been approved by the Food and Drug Administration and other regulatory agencies (Roberts et al. 2020; Shen and Corey 2018; Sharma et al. 2014).

Among ARCAs, the antisense and nucleic acid-based drugs strategy has been investigated only in FRDA and AT. In fact, Li and collaborators have first demonstrated that directing duplex RNAs or single-stranded locked nucleic acid oligonucleotides on the GAA-repeated intronic regions of the *FXN* gene significantly increase the expression of the mRNA and the protein in patient-derived fibroblasts (Li et al. 2016). They have proposed an interesting mechanism where the synthetic interfering oligonucleotides could act on the genomic DNA by blocking the interaction with R-loop and re-establishing a more sustainable level of transcripts. They have then tried to optimize their ASOs by modifying various chemical groups to enhance the specificity and stability of their approach (Li et al. 2018). The same team has then reinforced their previous observations by showing similar activation of FXN in iPSC-derived human neurons that have been electroporated with duplex RNA and ASOs (Shen et al. 2019). Similarly, Du and collaborators have worked with antisense morpholino oligonucleotides targeting three mutations in the *ATM*

gene and effectively restoring the normal splicing in cells isolated from AT patients (Du et al. 2007). Meanwhile, various types of autosomal dominant spinocerebellar ataxias were subject to more advanced preclinical studies using ASOs and animal models (Moore et al. 2017; Niu et al. 2018; O'Callaghan et al. 2020; Paulson et al. 2017; Scoles et al. 2017; Toonen et al. 2017). If successful, this technology could be eventually transposed to ARCAs, as they also are expansion diseases. A phase 3 clinical trial is still ongoing for the use of an exon-shipping ASO, namely Nusinersen, to treat children affected by spinal muscular atrophy (Chiriboga et al. 2016). Hence, the study has shown successful outcomes on survival and improvement of motor function, indicating the benefits and safety for potential administration of this group of drugs in other severe diseases.

An alternative molecular approach to ASOs is to use short hairpin RNA (shRNA), small interfering RNA (siRNA), or microRNA (miRNA) as RNA-based silencing tools. For instance, Shen and colleagues have demonstrated an increase in *FXN* expression using single-stranded siRNA in fibroblasts derived from FRDA patients (Shen et al. 2018). As described above with ASOs, these types of methods of interference have been well studied for dominant forms of ataxias and could be applied to ARCAs (Miyazaki et al. 2016; Nobrega et al. 2019; Ramachandran et al. 2014). Interestingly, modified adeno-associated viruses (AAV) or lentivirus have been used to deliver the appropriate machinery in animal models. Taken together, nucleic acid-based treatments display secure and promising preclinical outcomes for future human applications.

12.2 Gene Therapy and Genome Editing

Gene therapy and the CRISPR-Cas9 system offer a powerful and innovative strategy to correct a specific mutation or to modify the expression of a given gene for future clinical applications (Ran et al. 2013). The CRISPR-Cas9 technology has been adapted from bacterial immune defenses against viral infection to an effective human genome editing molecular tool. Thereupon, CRISPR has officially entered a human clinical trial for the first time (NCT03399448), which is a critical step for the future of humanity and life-threatening diseases (He 2020; Gillmore et al. 2021). Early preclinical data on the use of the technology are reassuring and point out that this novel technology is safe and durable. As revealed by several recent reports, FRDA and Cas9-based experimental therapies have been widely studied in vitro and in vivo. It has been previously demonstrated that delivering the CRISPR-Cas9 machinery into fibroblasts derived from two different FRDA mouse models has successfully removed the GAA intronic repeats (Ouellet et al. 2017). Rocca and her colleagues have re-established the wild-type expression of the *FXN* in FRDA patient-derived $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) by excising the expansion with a combination of the Cas9 nuclease and a pair of guides RNA (Rocca et al. 2020). While genome editing has slightly delayed cell proliferation for a brief duration, the corrected HSPCs exhibited no cytotoxic effect and have

been able to correctly differentiate when transplanted in an immunosuppressed mouse model. Furthermore, a novel 3D organoid model, harboring marked neuropathological phenotypes of FRDA, has been developed and then the CRISPR-Cas9-induced deletion of the GAA-expanded regions has rescued some of the disease-associated deficits (Mazzara et al. 2020). In a wider spectrum of gene therapy, intravenous AAV delivery of the full-length human *FXN* gene in conditional mouse model depleted in frataxin has interestingly reverse cardiac damages (Perdomini et al. 2014). Similarly, reintroduction of the *ATM* gene using an optimized hybrid viral vector has been performed in AT human cells and Atm knockout mice (Cortes et al. 2008). For now, we must consider genome editing and the CRISPR-Cas9 system as experimental tools and are not yet therapies for ARCAs. Only future will tell.

12.3 Epigenetic

Since clear epigenetic changes have been involved in the process of *FXN* gene silencing, epigenetic-based drugs, such as DNA demethylating agents (Ouyang et al. 2018; Chiurazzi et al. 1999), histone deacetylase inhibitors (HDAC) (Gottesfeld et al. 2013; Chan et al. 2013; Lufino et al. 2013), and histone methyltransferase inhibitors (Punga and Buhler 2010; Sandi et al. 2013), are of particular interest by many research teams (Sandi et al. 2014). Meanwhile, treatments with small molecules from the class I HDAC inhibitors, such as 2-aminobenzamide, have proven to be effective in restoring some frataxin expression in cells derived from FRDA patients and transgenic mouse models of FRDA (Rai et al. 2008, 2010; Sandi et al. 2011; Herman et al. 2006). In addition, Chan and his collaborators have demonstrated that nicotinamide, a class III HDAC inhibitor, can significantly increase histone acetylation at the *FXN* locus in FRDA primary human cells and in a FRDA mouse model, which in turn increase frataxin mRNA and protein levels (Chan et al. 2013). Alternatively, a modified version of the Cas9 protein, which has been designed to target a specific promoter region and enhances transcription by altering the chromatin organization, could also be considered as a future FRDA therapy (Hilton et al. 2015; Black et al. 2016). More research is needed to check the feasibility and security of this approach. CRISPR has not been tested yet in humans.

12.4 Stem Cells

Stem cell transplantation seems to be a noteworthy avenue for treating a large spectrum of spinocerebellar ataxia. Jones and collaborators have shown that intrathecal injection of wild-type mesenchymal stem cells (MSCs) in an FRDA mouse model led to beneficial effect on reducing motor deficits (Jones et al. 2015). MSCs can correctly differentiate and migrate at DRG in the spinal cord, which then increased

neuronal survival and protection 5 months post-intervention. It has been previously demonstrated that a single injection of HSPCs into the tail vein of the FRDA mouse model can restore the disease-associated symptoms (Rocca et al. 2017). A similar study has used transplantation of bone marrow cells instead of HSPCs, which has also improved motor functions in vivo *(*Kemp et al. 2018*)*. In addition, transplanted bone marrow cells have stably integrated the host's DR66G, spinal cord, and cerebellum, and normal expression of FXN increased the cellular defense against oxidative stress. Alike, bone marrow cell injection in a mouse model deficient in Atm has re-established some AT-associated phenotypes (Van Hoesen et al. 1972). In an impressive way, allogeneic stem cell transplantation in children affected with AT offer promising results for future treatment (Ussowicz et al. 2013; Beier et al. 2016; Ussowicz et al. 2018).

Dongmei and his collaborators have directed a nonrandomized clinical trial where SCA and multiple system atrophy-cerebellar patients were intrathecal-injected with wild-type umbilical cord-derived MSCs (Dongmei et al. 2011). They conclude that this type of intervention is safe, stable in time and can postpone principal diseases-associated symptoms. Similar conclusions have been observed by another research team, which has established comparable parameters for their clinical study (Jin et al. 2013). Likewise, a pilot open-label phase 1/2 trial for the transplantation of allogenous adipose tissue-derived MSCs in 6 SCA3 patients has given encouraging outcomes in terms of security and integration (Jin et al. 2013). However, a recently published systematic review and meta-analysis conducted with the clinical trials described above have concluded that there is no statistical correlation between patient recovery and stem cell transplantation (Jin et al. 2013). Nevertheless, a randomized phase 2 clinical trial will soon be managed, in a larger cohort of 45 patients, for the usage of allogenous umbilical cord-derived MSCs for treating various forms of dominant SCA (NCT03378414). A careful follow-up of this new study remains evident in order to obtain clear indications on the relevance of this type of intervention. Alternatively, a combination of gene editing and autologous stem cell engraftment would be of particular interest as a more futuristic approach to treat inherited ataxias.

12.5 Vaccines

Although being a preclinical approach in ARCAs and X-linked degenerative ataxias, vaccines have recently caught more attention worldwide. The lately approved vaccines against the novel coronavirus have proven to be safe and effective, and open the way to a plethora of clinical applications (Polack et al. 2020). The idea of exploiting gene-based and peptide-based vaccines to treat neurodegenerative disorders and other complex diseases portends great hope for the future. Of particular interest, messenger RNA vaccines have been under clinical for quite a time now, especially in the field of oncology (Pardi et al. 2018; Miao et al. 2021). The pandemic has clearly accelerated the processes leading to the approval for human use.

For years, vaccine and immunotherapeutic strategies have also been largely studied in neurodegenerative diseases, especially in AD and Parkinson's disease (PD) (Kudrna and Ugen 2015). As an example, a phase 2 trial for the use of a more conventional active peptide vaccine directed against β-amyloid protein has been currently completed in AD patients and has indicated reassuring results in terms of safety and immunogenicity (Novak et al. 2021). Viral vectors and naked DNA gene-based technologies present distinct advantages over peptide- and protein-based vaccines but also have some limitations to consider. However, such strategies have only been conducted preclinically in AD mouse model (Hara et al. 2004; Mouri et al. 2007; Davtyan et al. 2014; Chen et al. 2013). Meanwhile, a research team has demonstrated an exciting proof-of-concept for the potential use of viruses for the delivery of the wild-type *FXN* gene in FRDA patients (Khonsari et al. 2016). They manage to permanently incorporate the full gene in human and mouse FRDA fibroblasts using lentivirus particles. High-*FXN* expression has no significant toxic effect, re-establishes a certain mitochondrial iron homeostasis, enhances the antioxidant response, and may reduce genomic instability. Of course, in vivo studies must be conducted to evaluate the effectiveness of such a strategy on a living organism. Subsequently, viral-based vaccines could be exploited as a shuttle to transport a large spectrum of therapeutic drugs, such as ASOs and the CRISPR-Cas9 machinery, at a desired cerebral region or a specific cell type.

13 Conclusion

ARCAs are a category of rare neurological conditions with a wide range of clinical and genetic variability. The focus of this chapter was to discuss basic and novel aspects about the Genetics of autosomal and X-linked progressive (or degenerative) ataxias, emphasizing on prospective therapies. Currently, thanks to next-generation sequencing, the number of genes known to cause ataxia is growing every month (Renaud et al. 2020). Although the access to molecular analysis remains limited, the progress of knowledge about the genetics of ARCAs is a first step toward improving the lifestyle for patients and potentially disrupting the progression of the diseases. Future exploration into nucleic acid-based drugs, gene therapy, genome editing, and stem cells could be useful to identify further therapy techniques. Advances like vaccines for other neurodegenerative conditions, particularly AD and PD, hold promising long-term effective approaches for ARCAs. Also, various studies are pursued to discover treatment options for FRDA, by far the most common ARCAs. These studies could be indicators for other rare ataxias with similar pathophysiology as FRDA.

One of the barriers to develop new strategies is the lack of clinical rating scales for many ataxias, leading to numerous undiagnosed cases. The genetic heterogeneity, the wide range of symptoms, and the overlap with other conditions can also complicate the diagnosis of RCAs. Furthermore, the important variability of ARCAs makes it difficult for medical professionals to successfully manage the symptoms. Distinct therapeutic methods may be necessary for each condition and collaborative

efforts will certainly help the elaboration of large clinical data and genomic data registries (Traschutz et al. 2021). Also, even though many international panels of experts have tried to develop a nomenclature and classification system for ARCAs, a final consensus still needs to be reached. As more evidence become available rapidly, it can be complex to maintain an updated classification. The overall aim is still to discover more about the pathophysiology of these severe and complex diseases. Indeed, the physiopathology for many ataxias remains unknown, which complicates the development of specific and effective strategies. However, the current state of technology and the various ongoing studies are reasons to be optimistic about the future of ARCAs treatment.

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Seeking Therapies for Spinocerebellar Ataxia: From Gene Silencing to Systems-Based Approaches

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Abstract Recent progress has led to nucleotide-based gene silencing strategies as a potential therapy for spinocerebellar ataxia type 3 (SCA3) and other hereditary ataxias. But recent setbacks for antisense oligonucleotide (ASO) therapy in another CAG repeat disease, Huntington's disease (HD), remind us of the importance of broadening the search for potential routes to disease-modifying therapies. Here we review alternative approaches. We begin by introducing some of the complexities and nuances of SCA3 that can help guide therapeutic strategies. We then review the myriad biological pathways that are potentially druggable in SCA3, and the current use of genetic and small molecule screens to identify targets and possible therapeutic agents. Given the importance of employing model systems in which the disease gene and protein are assessed at physiological concentrations in the human genomic context, we discuss the emerging importance and challenges of using human stem cells to study disease mechanisms and test therapeutic targets and novel pharmacologic agents. We conclude by considering SCA3 and related SCAs beyond bio-

For inclusion in: *Trials of Cerebellar Ataxia: From Disease Models to Therapeutics.*

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_6

chemical pathways and as diseases of impaired connectivity, emphasizing the largely untapped potential of modulating neuronal activity and brain connectivity for possible disease-modifying therapies.

Keywords Repeat expansion · Stem cells · Organoids · Dysfunctional circuitry

1 Introduction

Now is an exciting time in SCA3 research. Decades of hard work by many groups to understand disease mechanisms and develop treatments could soon lead to rationally based disease-modifying therapies for this progressive and fatal neurodegenerative disease. As we write this in 2022, at least one company is performing a human clinical trial of nucleotide-based gene silencing therapy as a potential disease-modifying treatment in SCA3. As contributors to the preclinical work establishing gene silencing as a treatment strategy (McLoughlin et al. 2018), we fervently hope these gene-directed therapies find their way into the clinic. The role of nucleotide-based gene silencing therapies in SCA3 is well covered in Dr. McLoughlin's chapter in this collection (McLoughlin 2022). Here, we focus instead on other approaches to therapy. We do not know where the best therapies will come from in SCA3, and researchers must adopt a broad perspective in order to capture the most effective agents (Costa 2020; Da Silva et al. 2019). Doing so requires that scientists recognize the complexities of SCA3 disease pathogenesis and consider alternative approaches to ameliorating symptoms and altering disease course. It is also worth considering, even at the basic science stage, how addressing these alternative mechanisms may be tracked to develop biomarkers of disease and target engagement.

What follows represents a selective review of the current state of therapeutic development for SCA3, emphasizing research published in the past 5 years. We begin by introducing some of the complexities and nuances of SCA3 that can help guide therapeutic strategies. We then review the use of genetic and small molecule screens to identify targets and potential therapies, discuss the importance and challenges of using human stem cells to study disease mechanisms and test therapeutic targets, and conclude by considering the SCAs as dysfunctional circuitry and reviewing the largely untapped potential of modulating neuronal activity and brain connectivity.

2 Complex Issues in SCA3 to Consider as Therapies Are Sought

2.1 SCA3 Is Dominantly Inherited, but Elements Beyond Toxic Gain of Function Likely Contribute

Like other SCAs, SCA3 is an autosomal dominant disorder in which a single copy of the disease gene harboring the CAG repeat expansion causes disease (Paulson and Shakkottai 2020; Costa and Paulson 2012). In rare individuals carrying two expanded alleles, the disease is more severe with symptoms beginning early in life (Shang et al. 2018). An overwhelming preponderance of evidence from model systems and human disease tissue supports the view that the polyglutamine expansion in the disease protein ATXN3 drives one or more toxic effects in the nervous system (Paulson et al. 2017; McLoughlin et al. 2020). In other words, toxic properties conferred on the gene and encoded protein (so called "gain of function") drive the propagation of the disease. Moreover, the gene itself does not appear to be essential since mice lacking *Atxn3* appear essentially normal. It should be noted that while the exact interactions and dependencies of wild-type ATXN3 and polyglutamine-expanded ATXN3 are not fully understood, there is little evidence that loss of gene function is a major contributor to human disease. Thus, unlike in Huntington's disease (HD), where the gene protein is essential for early brain development, current antisense nucleotide-based approaches to reduce ATXN3 expression likely do not suffer from concerns that marked reduction in the ATXN3 protein itself will be deleterious.

And yet a contribution of loss of gene function to disease has not been formally excluded in SCA3. In a diseased brain, ATXN3 tends to concentrate in the nucleus of neurons, disappearing from the cytoplasm where it normally is more abundant. Potentially, this "loss" of ATXN3 from the cytoplasm contributes to disease pathogenesis. Moreover, recent studies reveal retinal involvement in SCA3 and a role for ATXN3 in the retina (Toulis et al. 2020, 2022), where the loss of the protein perturbs retinal structure and highlights a role for ATXN3 in regulating cilia and phagocytosis, both of which are fundamental to photoreceptor function. Further data indicate that ATXN3 modulates molecular features in certain cancers and participates in DNA repair (Chakraborty et al. 2020; Gong et al. 2021; Herzog et al. 2020; Zhuang et al. 2021). Accordingly, we should be mindful of the potential for deleterious effects of gene-silencing strategies that reduce ATXN3 levels too effectively and therapeutics targeting ATXN3 itself should be developed and analyzed with careful consideration of function of the native protein as well.

2.2 SCA3 Is a Neurodegenerative Disease, but Neurons Are Not the Only Involved Cell Type

The accumulation of ATXN3 nuclear inclusions in neurons of SCA3 disease brain, coupled with the fact that specific brain regions undergo profound atrophy in SCA3, placed attention on neurons as the principal cell type affected in SCA3. Recent studies, however, point to oligodendrocytes as an unexpected major contributor as well (Schuster et al. 2022). In that work, transcriptional changes in a mouse model of SCA3 revealed oligodendrocytes as the cell population most affected early on. While it remains to be seen whether ATXN3-mediated effects in oligodendrocytes drive disease processes, it will be important that therapeutic strategies not be focused solely on neurons lest nonneuronal populations prove paramount in targeting. Lessons might be learned from Alzheimer's disease, where an intense early focus on neurons delayed recognition of the critical disease contributions of astrocytes and microglia. In SCA3, we now know that oligodendrocytes are likely important, and recent data suggest that microglia are involved as well (Campos et al. 2022). As inflammation is proving to be an important contributor to other neurodegenerative diseases, it is critical that we define potential direct roles in SCA3 for microglia and astrocytes in disease pathogenesis. Finally, as we consider the roles of polyglutamine-expanded ATXN3 in various cell types, it behooves us to evaluate the levels and function of wild-type ATXN3 in response to the disease state, as this may provide insight into future treatment effects.

2.3 Proteotoxicity of the ATXN3 Disease Protein Is Important, but Not the Only Contributor to Disease

The CAG repeat expansion in SCA3 encodes an elongated stretch of the amino acid glutamine in ATXN3. Studies ranging from recombinant protein in vitro to cell-based models and various animal models of disease consistently have shown that expanded polyglutamine-containing ATXN3 is prone to aggregate and form intracellular inclusions in select brain regions and cell populations (reviewed in Costa and Paulson 2012; Paulson et al. 2017). Similarly, polyglutamine-containing disease proteins in SCAs 1, 2, 6, 7, and 17 also aggregate when the disease protein contains a polyglutamine expansion (Paulson et al. 2017). Accordingly, the prevailing view is that the primary toxic species in SCA3 is the ATXN3 protein containing the expansion. Understandably, then, mutant ATXN3 has been the target of many strategies to define disease-modifying therapies. That said, some evidence in model systems suggest toxicity at the RNA level occurs with higher expansion sizes (Li et al. 2008), and GC-rich repeat expansions are prone to undergo repeat-associated non-methionine (RAN) translation, in which protein can be translated in all three reading frames across the repeat leading to polyserine and polyalanine RAN products as well as the "expected" polyglutamine (Cleary et al. 2018). Evidence is

building for RAN translation in Huntington's disease (Cleary et al. 2018) and SCA8 (Perez et al. 2021) and is hinted at in SCA31 (Ishiguro et al. 2021), but remains limited in the more common CAG repeat expansion SCAs. One advantage of nucleotide-based gene silencing strategies is that they will reduce levels of both the RNA transcript and the disease protein. As a result, potential toxicity from RNA effects or RAN translation would also be mitigated. Therapies focused on the ATXN3 protein, or affected pathways downstream of the polyglutamine-expanded protein, would fail to address these potential upstream toxic effects.

2.4 SCA3 Affects the Brain, but Little Is Known About Disease in Other Organs

SCA3 is unquestionably a neurodegenerative disorder. Hence, effective therapies must penetrate the brain or be directly delivered to the nervous system. Clinically, the evidence for significant organ involvement beyond the central and peripheral nervous system is limited. The disease protein, however, is expressed ubiquitously. Thus, there may be subtler subclinical effects associated with the expression of the disease gene that have been missed to date. Retinal involvement in SCA3 is a recently described example (Toulis et al. 2022). And many SCA3 patients develop significant peripheral neuropathy that might not be fully addressed by a CNS-directed therapy. Accordingly, therapies such as orally delivered small molecules that can act throughout the body have a potential advantage over therapies that are directly delivered to the CNS, provided that the orally delivered compound displays favorable pharmacokinetics in the nervous system.

2.5 The ATXN3 Disease Protein Maybe Small, but Its Function Is Complex and Far-Reaching

ATXN3 is a specialized deubiquitinase that preferentially cleaves longer poly-ubiquitin chains (reviewed in Costa and Paulson 2012). A relatively small protein, ATXN3 readily moves in and out of the nucleus and participates in diverse ubiquitin-dependent processes at many places in the cell. Its various functions include working with ubiquitin ligases to modulate ubiquitin chain composition, regulating aggresome production, participating in DNA repair processes, and modulating autophagy and endocytic processes (Costa and Paulson 2012; Chakraborty et al. 2020; Dantuma and Herzog 2020; Rosselli-Murai et al. 2020; Zeng et al. 2020). Some properties of the protein suggest that ATXN3 is a unique deubiquitinase that participates in protein quality control pathways (e.g., ubiquitin-proteasome system and autophagy) that are themselves perturbed in SCA3. Precisely how the deubiquitinase activity and diverse functions of ATXN3 are perturbed in disease remains a

work in progress. In principle, its deubiquitinase activity represents a therapeutic target, but currently, we do not know if the enzyme activity of polyglutamine-expanded (mutant) ATXN3 is deleterious or beneficial compared to its native function and may have wide-reaching ramifications in cellular proteostasis. A potential negative consequence of inhibiting the enzyme activity of ATXN3 is that it could produce an avid ubiquitin-binding protein that lacks the ability to cleave the ubiquitin chains it binds; essentially the protein could act like a dominant-negative isoform on client proteins with potentially deleterious effects.

2.6 Studies of Overexpressed or Transgenic ATXN3 Have Shed Important Light on Disease Mechanisms but May Not Mirror the Human Disease State

Most of the experiments providing insight into SCA3 disease mechanisms have relied on high-level expression of the disease protein in cellular and animal models, and often not from the endogenous locus. Overexpression of the disease protein accelerates the appearance of molecular and neuropathological phenotypes in short-lived model systems, whereas expression of the protein at endogenous levels has more subtle effects that can become impractical in a laboratory setting. But as helpful as overexpression systems have been, they run the risk of leading to artifacts and spurious findings that do not mirror the physiological state of cells in humans with SCA3. Similarly, relying on transgenic expression models poses possible confounding factors of altered regulation. Particularly as scientists search for genes or compounds that regulate levels of SCA disease proteins, it will be important to screen for such regulatory factors in model systems that mimic the native physiological levels and actions of the disease gene and protein. Doing so poses its own challenges, but it avoids the hazard of focusing on regulatory factors that might simply work to mitigate an overexpression artifact. The emergence of human stem cells derived from SCA3 patients or embryos (discussed further below) allows for the examination of the disease protein and its effects under the most germane physiological conditions.

2.7 ATXN3 Maybe the Obvious Target in SCA3, but Targets Beyond and Downstream of ATXN3 Also Need to Be Explored

Observed effects beyond the polyglutamine expansion suggest additional, potentially targetable pathways in SCA3. For example, single- and double-strand DNA breaks accumulate in cellular models of HD (Illuzi et al. 2008), and overexpression of DNA repair enzymes can ameliorate the disease phenotype in mouse models

(Enokido et al. 2010). In SCA3, ATXN3 promotes the activity of the 3′ phosphatase and 5′ kinase PNKP (Chatterjee et al. 2014) and has been linked to nonhomologous end-joining (NHEJ) repair of double-strand DNA breaks (Chakraborty et al. 2020). The DNA repair pathway may also offer a route through which to target repeat expansion instability as small molecules that contract CAG repeats have been published (Nakamori et al. 2018). Epigenetic mechanisms have also been implicated in SCA3 as DNA methylation within the *ATXN3* promoter inversely correlates with the age of onset and intergenerational repeat instability (Wang et al. 2016).

2.8 Most Research in SCA3 Has Focused on Disease Effects at the Cellular Level, but Network-Level Effects Remain Understudied

Addressing the widespread reaches of polyglutamine-expanded ATXN3 demands a comprehensive approach to improve patient care. While SCA3 is clearly a systemic disease with extra-CNS complications, even within the nervous system we must expand our disease framework. We have greatly improved our understanding of the biochemical mechanisms at play in SCA3 and the other SCAs, but this has not led to novel drugs beyond gene-silencing therapies, as mentioned above. It is therefore equally valuable to consider SCAs as diseases of impaired network connectivity. We are in a unique position to learn from the success of deep brain stimulation (DBS) in Parkinson's disease (PD) and essential tremor (ET) as a demonstration of the viability of electrophysiological perturbation for symptomatic management. A pharmacological approach with specificity against individual ion channels offers the opportunity to elicit similar therapeutic effects without surgical intervention.

3 Screens to Identify Targetable Pathways for Potential Therapy

On the one hand, the dominant-toxic nature of disease pathogenesis in SCA3 conceptually simplifies the route to therapy: agents that can reduce identified toxic species have strong therapeutic potential. On the other hand, many factors conspire to make the search for SCA3 therapies more complicated, including the chronicity of disease; diverse functions of the disease protein, including participation in stress pathways that are themselves implicated in disease; involvement of multiple cell types; and, the dynamic nature of the underlying mutation, a variably sized repeat expansion that could have deleterious effects at the DNA, RNA, and protein levels. Many researchers have successfully interrogated specific cellular pathways (e.g., molecular chaperones and autophagy) as potential routes to therapy, and nucleotide-based strategies that directly target the SCA3 disease gene have seen tremendous

progress recently (see McLoughlin 2022). Here we focus instead on small-molecule and genetic screens that have taken a more unbiased approach to identify compelling therapeutic agents. The number of such screens to date is limited, partly because the development of efficient and reproducible screening platforms takes considerable time and effort. With the advent of clustered regularly interspaced short palindromic repeat (CRISPR)-based approaches that enable rapid full-genome screens to find regulators of predefined readouts (say, ATXN3 levels) and the emergence of human stem cell lines harboring trackable epitope tags engineered into an endogenous gene of interest, we expect that the range, depth, and quality of screens for potential SCA3 therapies will ramp up.

Figure 1 highlights the many biological factors that could be modulated to alter levels of ATXN3, specific functions, or downstream effects tied to the protein. Arguably all are worth pursuing, and in many cases, promising initial data already support further investigations. These include boosting autophagy, enhancing chaperone activity, inhibiting specific protein-protein interactions, and blocking the proteolytic cleavage of ATXN3 that produces putative toxic fragments (recent examples

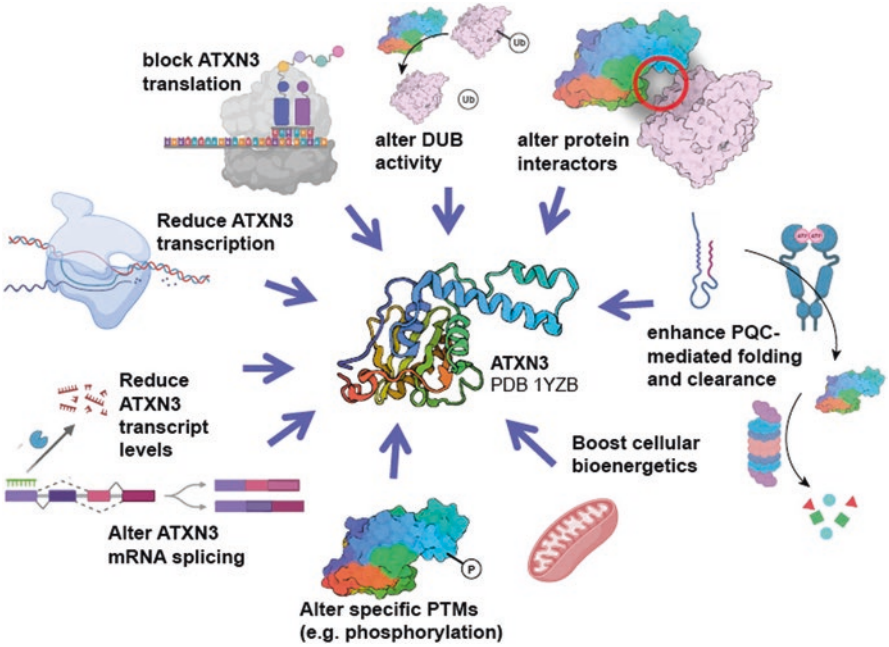

Fig. 1 Biological routes to potential disease-modifying treatment in SCA3. Cellular levels and activity of the disease protein ATXN3 are likely regulated by numerous biological pathways, illustrated here. Unbiased genetic and small molecule screens can identify genes or compounds that alter ATXN3 concentration, subcellular localization, or deubiquitinase activity, each of which may influence the toxicity of the disease protein. Alternatively, existing compounds or candidate genes implicated in specific pathways can be directly tested for disease-modifying effects. Current nucleotide-based gene silencing approaches act by reducing ATXN3 transcript levels

include Lee et al. 2021; Robinson et al. 2021; Vasconcelos-Ferreira et al. 2022). Many such studies were driven by a candidate target assessment and not linked to larger-scale screens. As outlined below, published screens to date have been performed to find compounds or genes that reduce levels of ATXN3 or reduce the toxic effects of ATXN3. We again stress the need to evaluate wild-type and polyglutamine-expanded ATXN3 separately and together to better understand potential treatment effects. Ideal screens will incorporate assays by which total and mutant ATXN3 levels can be measured in response to any cellular perturbation.

Using an immortalized nonneuronal cell line that stably expresses luciferase-tagged ATXN3, Costa and colleagues (Costa et al. 2016) screened a small-molecule library largely containing FDA-approved drugs. A series of secondary screens in other cell lines and in cerebellar brain slices from a SCA3 mouse model identified the atypical antipsychotic aripiprazole as an ATXN3 modulator. Short-term exposure of SCA3 mice to this drug resulted in a marked reduction of aggregated ATXN3. The mechanism by which aripiprazole acted on ATXN3 was unclear, particularly since the starting cell line, derived from HEK293 cells, was not known to express neurotransmitter receptors by which the drug might work. A recent follow-up study in a nematode model of SCA3 suggests that aripiprazole acts through dopamine and serotonin receptors to reduce the motor effects of ATXN3 (Jalles et al. 2022). To date, aripiprazole has not been tested in a clinical trial of patients with SCA3. Because some SCA3 patients develop parkinsonism and long-term exposure to aripiprazole can elicit a movement disorder among other side effects, aripiprazole usage in SCA3 will have to be monitored closely.

This same cell-based screening platform was used in a druggable genome screen to identify genes that regulate levels of ATXN3 (Ashraf et al. 2020). In this screen, 317 candidate genes were identified and 100 were selected for validation, 33 of which were confirmed in multiple secondary assays. Of these, 15 were independently validated in separate cell lines as modulators of ATXN3 levels. Further analysis in a Drosophila model of disease confirmed the effects of several genes in vivo, and one gene, *FBXL3*, was shown to reduce ATXN3 levels through ubiquitin-dependent processes. Additional analyses revealed a molecular network linked to tumor necrosis factor/nuclear factor-Kappa B and to the extracellular signal-regulated kinases 1 and 2 (ERK1/2), which are pharmacologically targetable. Further analysis of these identified genes in mouse models and human disease tissue will be required to confirm their relevance to human disease. Limitations to this screen include the fact that an immortalized nonneural cell line was used that overexpresses ATXN3. Despite these limitations, the screen demonstrated the feasibility of further genetic screens in more physiologically relevant cellular and animal models.

Maciel and colleagues performed a small molecule screen in a nematode (*Caenorhabditis elegans*) model of SCA3 that displays ATXN3-mediated immotility (Teixeira-Castro et al. 2015). From this screen, the antidepressant citalopram surfaced as a drug that could rescue motility. Moreover, a follow-up study in a mouse model of SCA3 showed that citalopram reduced ATXN3 aggregation (Ashraf et al. 2019). Thus, citalopram, a widely prescribed antidepressant, holds promise as

a potential disease-modifying therapy in SCA3. The small size, short life cycle, and genetic tractability of the nematode make it a strong organismal platform for genetic and compound screening. Leveraging these advantages, Maciel and colleagues conducted further studies in the nematode that highlight the importance of serotonin signaling as a modulator of ATXN3 proteotoxicity, and support future human clinical trials of serotonin-acting agents such as specific 5-HT1A agonists (Pereira-Sousa et al. 2021).

The nematode model of SCA3 has proved useful in other genetic and compound screens. For example, a screen of nearly 4000 compounds, many FDA-approved, revealed five lead compounds that restored motility in the model (Fardghassemi and Parker 2021). Three of these compounds were found to modulate a key transcriptional regulator of autophagy, TF EB/HLH-30. This screen not only highlights the relevance of autophagy to SCA3 (Fig. 1), in part as a potential clearance mechanism for mutant ATXN3, but also supports the use of specific autophagy-regulating small molecules as possible therapeutic agents. In another recent study employing this nematode model, an RNA interference (RNAi) screen of nearly 400 transcription factor genes identified one with apparent neuroprotective activities, FKH-2/FOXG1 (Fardghassemi et al. 2021). The mechanism of neuroprotection by this transcription factor has not yet been defined.

The above studies underscore the need for additional large-scale screens to identify therapeutic targets and possible therapeutic agents. What is currently lacking are human stem cell-based screening platforms that can capture changes in ATXN3 when expressed at endogenous levels. CRISPR-based genetic screens, coupled with automated cell sorting that employs a robust, sensitive anti-ATXN3 antibody or ligand to quantify ATXN3 levels, could capture genes that up- or down-regulate ATXN3. A suitably tagged form of ATXN3, genetically engineered into the endogenous *ATXN3* locus of human stem cells, might enable a physiologically relevant screening platform for small molecules, provided that the signal to detect ATXN3 is sensitive and specific.

4 Human Stem Cells as a New Tool for Mechanistic and Translational Studies

Animal models have proven to be an excellent platform for assessing pathogenic mechanisms and testing candidate therapeutics for SCA3 and other CAG repeat expansion diseases including HD. Nevertheless, these models have limitations. Human stem cells provide a compelling opportunity to expand basic and translational investigations of SCA3 and other repeat expansion diseases. Favorable characteristics include the fact that the human disease gene is expressed at endogenous levels from its native locus within the full human genomic context. Here we review the use of stem cells in SCA3. Because HD has been more extensively examined

using stem cells than any of the SCAs, we also refer to the more robust HD literature to highlight the potential of stem cells.

Both human induced pluripotent stem cells (iPSCs) and human embryonic stem cells (hESCs) are beginning to play significant roles in the study of various repeat expansion diseases. iPSCs are generated by reprogramming adult somatic cells (e.g., skin fibroblasts and peripheral blood mononuclear cells) to an embryonic-like state. Yamanaka originally demonstrated that iPSCs could be derived from fibroblasts by transducing various transcription factors (Oct4, Sox2, Klf4, and c-Myc) into murine embryonic and adult fibroblasts (Takahashi and Yamanaka 2006). More recently, nonintegrative gene delivery approaches have also been established (Al Abbar et al. 2020). The resultant iPSCs, in turn, can be differentiated into various cell types, permitting cell type-specific analyses. The intermediate pluripotent state can also be skipped through direct reprogramming, which allows adult somatic cells to transition directly from one lineage to another. For example, Ambasudhan et al. (2011) used this approach to generate functional neurons directly from adult human fibroblasts. In contrast, hESCs are not generated through reprogramming. As the name implies, hESCs are derived from the inner cell mass of blastocyst-stage embryos and can give rise to all somatic cell types in the embryo (Lee and Lee 2011).

Stem cells hold promise as model systems in which to discover potential therapeutic targets. For both SCA3 and HD, various nucleotide-based approaches (e.g., siRNAs, shRNAs, microRNAs, and ASOs) and small molecules have successfully reduced the mutant transcript and/or disease protein levels (Alves et al. 2008, 2010; Ashraf et al. 2019; Carroll et al. 2011; Costa et al. 2016; Estevez-Fraga et al. 2020; Komatsu 2021; McLoughlin et al. 2018; Moore et al. 2019; Sun et al. 2014; Tabrizi et al. 2019). While nucleotide-based gene silencing may lead to disease-modifying therapies for SCA3 and other dominantly inherited ataxias, ataxia researchers need to continue seeking and developing alternative therapeutic strategies. A promising avenue for uncovering such strategies is to identify the molecular mechanisms regulating mutant protein production, stability, and/or clearance as highlighted in Fig. 1.

Stem cells as a therapeutic strategy may also hold promise. In their review, Sivandzade and Cucullo (2021) explain that stem cells could either replace damaged cells with differentiated ones or promote an environment conducive to regeneration through neurotrophic support. Alterations to the CNS environment elicited by stem cells also might prevent damage to the remaining healthy neurons and glia. We caution that the field of regenerative medicine is relatively young and further advances are needed for stem cell therapy to enter the clinical setting as a treatment option for SCA3 and other polyglutamine expansion diseases.

For a variety of reasons, both iPSCs and hESCs are excellent tools for seeking and developing alternative therapeutic strategies. First, stem cells obtained from patients can recapitulate many disease-associated phenotypes, including transcriptional dysregulation, mutant protein aggregation, lysosomal dysfunction, and neuronal vulnerability (Cheng et al. 2013; Hansen et al. 2016; He et al. 2021; Jaworska et al. 2016; Jeon et al. 2012; Koch et al. 2011; Lu and Palacino 2013; Moore et al. 2019; Niclis et al. 2013; Tousley and Kegel-Gleason 2016). Second, stem cells can be differentiated into various somatic cell types, which enables the examination of

disease-specific cell type vulnerability. Third, stem cells express the disease gene of interest (e.g., *ATXN3* or *HTT*) at endogenous levels from the native locus, eliminating nonphysiological effects and spurious results that may be observed when disease genes are overexpressed. Fourth, in stem cells, signal transduction pathways that contribute to disease development and progression may be uncovered more readily. Finally, isogenic cell lines can be established as critical controls for stem cell lines and their generation is currently underway in SCA3. Previous research has revealed that single nucleotide polymorphisms in the genome of different patient samples can affect research results. The generation of multiple isogenic cell lines for a disease enables one to distinguish critical disease gene-associated findings from effects due simply to variations in genetic background. Two approaches can be used to generate isogenic pairs of cell lines. The first approach removes the mutation from patient-specific cells, while the second introduces the mutation into wild-type cells. Transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9 are frequently used as gene-editing tools and have successfully been employed to generate isogenic lines for SCA3 and HD (Dabrowska et al. 2020; He et al. 2021; Lu and Palacino 2013; Malankhanova et al. 2017, 2020; Ooi et al. 2019). These control lines are essential when performing compound screens or identifying differentially expressed genes and proteins that are associated with a disease. Not only can isogenic lines enable understanding of the contribution of the *ATXN3* CAG expansion to disease-linked cellular phenotypes compared to otherwise identical cells harboring a normal CAG repeat, scientists can also genetically increase the CAG repeat expansion to enhance the disease phenotype and better understand clinical heterogeneity and even the phenomenon of anticipation.

To date, numerous scientific questions pertaining to HD have been addressed in iPSCs and hESCs. Malankhanova et al. (2020) generated isogenic lines using HD-iPSCs reprogrammed from fibroblast clones. The resulting cells were differentiated into striatal medium spiny neurons, which are known to be selectively vulnerable in HD. While iPSC-derived neurons harboring the CAG-expanded *HTT* allele did not develop huntingtin protein aggregates, they did accumulate ultrastructural defects detectable by electron microscopy. The authors suggested these defects may occur early in the pathogenesis of HD, before aggregate formation. Niclis et al. (2013) compared two HD-hESC lines to a wild-type control line both in the undifferentiated state and during differentiation into forebrain neurons. The two HD-hESC lines had CAG-expanded *HTT* alleles of 37 repeats and 51 repeats, respectively. Whether as undifferentiated or differentiating cells, HD-hESC and wild-type lines were indistinguishable with respect to growth, viability, pluripotent gene expression, mitochondrial activity, and capacity to differentiate into neurons. Furthermore, the expression levels of genes known to be perturbed in HD were similar across the hESC lines. The authors did note, however, that neurons derived from HD-hESCs with the larger repeat expansion (51 CAG repeats) displayed elevated glutamate-evoked responses. These studies confirm that stem cells with a CAG-expanded *HTT* allele maintain pluripotent parameters and can differentiate into various somatic cells. In addition, the neuronal progeny may display phenotypes associated with HD.

In studies of SCA3, similar questions have been tackled using iPSCs and hESCs derived from patients. Koch et al. (2011) reported the first SCA3-iPSC line, which paved the way for the development of additional lines. For example, He et al. (2021) used CRISPR/Cas9-mediated homologous recombination to correct SCA3-iPSC lines; for each line, the abnormal CAG repeat expansion was replaced with a normal repeat length. Neurons derived from the SCA3-iPSC lines exhibited several phenotypic abnormalities: polyglutamine protein aggregates; decreased mitochondrial membrane potential and glutathione expression; and, increased reactive oxygen species, intracellular Ca2+ concentrations, and lipid peroxidase malondialdehyde levels. Importantly, neurons generated from genetically corrected SCA3-iPSC lines did not display these abnormal phenotypes. This study highlights the ability of isogenic lines to unveil phenotypes associated with the CAG-expanded *ATXN3* allele.

Moore et al. (2019) reported the first NIH-approved SCA3-hESC line, which recapitulated certain molecular features of human disease, most notably the production of aggregates. Their study highlighted the potential for SCA3-hESCs to function as a cell-based disease model. When SCA3-hESC cells were exposed to a validated anti-ATXN3 antisense oligonucleotide (ASO), the ASO reduced the expression of mutant ATXN3, reversing ATXN3 aggregation and aggresome formation in SCA3-hESCs. As discussed further below, whereas the phenotype of ATXN3 aggregation was spontaneously observed in the SCA3-hESC line, similar aggregation in differentiated SCA3-iPSC lines required that the cells be stressed through depolarization (Koch et al. 2011). Whether this discrepancy reflects a difference in the ability of iPSC versus hESC lines to mirror molecular features of disease will require further head-to-head comparisons of multiple iPSC and hESC lines harboring the same mutation.

There are numerous discrepancies with respect to protein aggregation in stem cell models of CAG repeat diseases. Most of the HD literature, for example, suggests that HD-iPSCs do not exhibit protein aggregation spontaneously or even after exposure to cellular stressors (e.g., hydrogen peroxide, 3-methyladenine, and repetitive exposure to glutamate) (Jaworska et al. 2016). Both Jeon et al. (2012) and Cheng et al. (2013), however, were able to trigger aggregate formation in stem cells by exposing them to a proteasome inhibitor. It is also unclear whether neurons derived from HD-iPSCs contain aggregates (Jaworska et al. 2016; Jeon et al. 2012; Malankhanova et al. 2020; Zhang et al. 2010). With respect to SCA3, several groups have reported excitation-induced aggregation in neurons, but not in iPSCs and other non-neuronal cells (Hansen et al. 2016; He et al. 2021; Jaworska et al. 2016; Koch et al. 2011). The literature pertaining to HD-hESCs is inconsistent (Lu and Palacino 2013; Niclis et al. 2009; Ooi et al. 2019). Lu and Palacino (2013) were able to trigger the formation of aggregates after transfecting cells with cDNA encoding HTT exon1 fragments with various polyglutamine lengths (Q23 for wild-type; Q73 and Q145 for HD). Otherwise, the literature suggests that HD-hESCs and neuronal progeny often do not develop HTT aggregates. In contrast, Moore et al. (2019) found that SCA3-hESCs developed protein aggregates in the absence of cellular stressors. The undifferentiated state and early passage number (or young age) associated with stem cells and neuronal progeny, respectively, may account for the absence of protein

aggregation in many of these studies (Ooi et al. 2019). Nekrasov et al. (2016) observed HTT inclusions in older (6-month-old) neurons derived from HD-iPSCs. Following intracerebral transplantation in mice, Jeon et al. (2012) found that neurons derived from HD-iPSCs contained aggregates when assessed 33 or 40 weeks later.

Stem cell-based research on HD and SCA3 over the past decade has not shed much light on somatic CAG repeat instability, which recently has surfaced as a potential contributor to disease pathogenesis (Paulson 2018). With respect to HD, most literature suggests that iPSCs do not exhibit repeat instability during long-term passaging (Camnasio et al. 2012; Jaworska et al. 2016; Jeon et al. 2012). Interestingly, Mattis et al. (2012) found that iPSC-derived neural stem cells (NSCs) displayed mild repeat instability following long-term passaging: by passage 26, the CAG-expanded *HTT* allele in one of the lines contained 118 repeats rather than 110. The CAG repeat length in SCA3-iPSCs appears to remain stable following long-term passaging and differentiation into neurons (Jaworska et al. 2016; Ou et al. 2016). Limited information is available for repeat stability in hESCs. Most studies suggest that, like HD-iPSCs, HD-hESCs do not exhibit repeat instability. Neuronal progeny, on the other hand, often have minor repeat instability (Niclis et al. 2009; Ooi et al. 2019). To date, repeat length instability in SCA3-hESCs following long-term passaging and differentiation has not been assessed rigorously. Potentially, high fidelity of DNA replication in stem cells may prevent repeat instability from occurring. Alternatively, the process of somatic repeat instability might require greater time and more accumulated cell divisions than is typically assessed in stem cell studies employing undifferentiated or differentiated cell populations (Ooi et al. 2019).

Evidence to date raises the intriguing possibility that iPSC and hESC lines differ in ways that would favor one versus the other for mechanistic and translational studies. Epigenetic differences are the most likely reason why the two cell types may differ (Narsinh et al. 2011). Direct comparisons of disease-relevant phenotypes (e.g., repeat instability, protein aggregation, and transcriptional changes) are needed to determine the relative value of each stem cell type as a disease model for SCA3 or other repeat expansion ataxias.

As two-dimensional cell culture is somewhat limited, three-dimensional (3D) organoids may offer new insights into the pathogenesis of SCA3 and other CAG repeat diseases. Organoids are generated through the aggregation of stem cells. When exposed to various signaling molecules, aggregated stem cells differentiate into multiple cell types. Through self-organization and self-renewal, these 3D structures mimic organ-specific cellular patterns and functions. In their review, Hou and Kuo (2022) highlight the strengths associated with CNS organoids, including their organ-like spatial cell arrangements and microenvironment. Unlike 2D cultures, the presence of multiple cell types with proper orientation and adjacency in CNS organoids promotes the occurrence of paracrine- and direct contact-mediated interactions similar to those that occur in vivo. Research involving 2D culture facilitated the discovery of cell-intrinsic mechanisms that promote disease development and progression. CNS organoids provide an opportunity to build on this understanding by examining pathological events in the context of neuronal networks. This system could reveal how repeat instability, aggregation propensity, and cellular

vulnerability vary between cell types. The use of organoids could also uncover early neurodevelopmental aspects of HD, SCA3, and other CAG repeat diseases, which remain understudied. Lastly, as the HD ASOs are thought to have been toxic due to high concentrations required to reach deep brain tissue, organoids offer an early screening step to evaluate such possible adverse effects.

Recent developments have enhanced the utility of CNS organoids as a model for neurodegenerative diseases. For example, distinct types of CNS organoids have successfully been established: cortical, striatal, midbrain, cerebellar, and motor neuron. The process of generating 3D structures can be divided into two sequential, induction steps. The first and second steps result in the formation of neuroepithelium and region-specific lineages, respectively. By modifying the signaling molecules present during the second step, the developmental patterning characteristic to a specific CNS region can be initiated (Bang et al. 2021; Hou and Kuo 2022). Fused region-specific brain organoids are also becoming more prevalent. For example, Chang et al. (2020) highlighted multiple studies that examine fused dorsal–ventral-patterned organoids as models for brain development. They point out that a caveat associated with CNS organoids is the lack of a circulatory system. Scientists have attempted to resolve this issue by transplanting CNS organoids into an in vivo environment. Another approach entails engineering hESCs that ectopically express erythroblast transformation specific (ETS) variant transcription factor 2 (ETV2) and therefore can form vascular-like structures. As summarized by Tidball et al. (2022), most methods for establishing CNS organoids result in 3D structures with multiple neural rosettes. These rosettes correlate with neural tube formation during embryonic development and, unfortunately, promote structural heterogeneity between organoids. To reduce this heterogeneity and enhance their biological relevance, Tidball et al. (2022) developed a protocol to establish CNS organoids with a single neural rosette organizing center.

The use of CNS organoids to study both SCA3 and HD is in its infancy. Depla et al. (2020) generated iPSC-derived cerebral organoids from a healthy control patient and used them to examine the viral transduction efficiency and distribution of rAAV5, a commonly used AAV serotype. The rAAV5 was engineered to deliver microRNA targeting *ATXN3* mRNA and was able to lower the expression of wild-type ATXN3 protein by 30%. The organoid literature pertaining to HD is more extensive. For example, Conforti et al. (2018) generated cerebral organoids using iPSCs derived from healthy and HD patients. When compared to the control organoids, the HD organoids exhibited defects in striatal and cortical fate differentiation, cytoarchitecture, and neuronal maturation.

Overall, as is true for mouse models, no one stem cell model system is likely sufficient when studying the underlying molecular mechanisms of HD, SCA3, or other SCAs. Each model system has strengths and weaknesses. For example, the process of generating iPSCs from somatic cells may result in the loss of epigenetic modifications that are critical for disease development and progression (Narsinh et al. 2011). Due to ethical reasons, it is more difficult to acquire hESCs compared to iPSCs. With respect to CNS organoids, the process of generating them is expensive and time intensive. Furthermore, this model system is still relatively new and in the process of being fine-tuned.

5 Impaired Connectivity as a Druggable SCA Target: Insight into Systems-Based Approach

While many therapeutic and mechanistic efforts remain focused on the root cause of SCAs, it has become apparent that analyzing downstream consequences of the genetic perturbation represents an important and unique opportunity for therapeutic intervention. Simply put, it makes physiological sense that correcting the genetic mutation in monogenic diseases like the SCAs should prevent future pathogenesis. This approach, however, is tempered by several caveats. The recent failure of two trials in HD using ASO therapy to reduce the expression of the disease gene gives insight into potential pitfalls (Kingwell 2021; Kwon 2021). Both trials against HD were stopped prematurely due to failure to meet primary endpoints, with one trial (Generation HD1) actually resulting in worsened patient outcomes. Which patients are most likely to benefit from genetic corrective therapy? And is targeting both wild-type and mutant isoforms ideal or prohibitive? In HD, it is clear that targeting both isoforms may be deleterious, though, as above, *ATXN3* knockout models do not suffer similar phenotypes as HD models do. Preclinical models using anti-*ATXN3* ASOs improve the motor phenotype, but it remains unclear if this will translate to human benefits. When is the best time to administer a gene-targeting intervention? What about patients that are already symptomatic? What about clinical heterogeneity in patients with similar *ATXN3* repeat lengths? While these outstanding questions are being explored in ongoing clinical research, it is worth considering how common downstream pathways might be exploited for therapeutic intervention. Such a strategy would ideally complement ongoing efforts at gene-based therapies. In this section, we examine SCA3 and other SCAs as diseases of impaired network connectivity that offer novel targets for intervention.

One major hallmark of SCAs is cerebellar Purkinje cell (PC) degeneration (Durr 2010; Paulson et al. 2017). It is also clear that impaired cerebellar Purkinje neuron firing is a shared feature in several SCA models. Moreover, alterations in PC spiking due to ion channel dysfunction occur concurrently with motor impairment and well before cell loss (Shakkottai et al. 2011; Hansen et al. 2013; Chopra et al. 2018). Recent work tied these changes in PC membrane excitability to reduced expression and activity of large-conductance calcium-activated potassium (BK) channels in SCAs 1, 2, and 7 (Dell'Orco et al. 2015, 2017; Chopra et al. 2018; Stoyas et al. 2020). In SCA1 transgenic mice, BK expression is transcriptionally repressed. Restoring BK channel expression or function rescues membrane hyperexcitability, improves motor dysfunction, and reduces PC degeneration (Dell'Orco et al. 2015; Chopra et al. 2018). These data suggest that ion channel dysfunction may drive neurotoxicity. Therefore, augmenting BK activity represents an attractive therapeutic strategy in SCA1 and possibly other SCAs, and is supported by recently published work (Srinivasan et al. 2022). An alternative strategy to restore appropriate PC spiking is combining chlorzoxazone and baclofen, which has proven successful in both a SCA1 mouse model and a limited open-label trial of SCA1 patients

(Bushart et al. 2021a). Despite not modulating BK function directly, this dual compound treatment strategy is expected to move into a Phase 2 Clinical Trial soon.

These findings raise several unanswered questions. For example, what specific channel should be targeted? As described above, BK (*KCNMA1*) channel activation is a compelling strategy for SCAs 1, 2, and 7. In contrast, SCA3 PCs are hyperexcitable and exhibit depolarization block (Shakkottai et al. 2011), and thus the irregular firing in SCA3 could theoretically be rectified by either a sodium channel blocker or a potassium channel activator. Recordings from Purkinje neurons in SCA3 transgenic mice, though, have demonstrated a clear reduction in voltage-gated potassium channel activation, specifically Kv1.6 (*Kcna6*) and Kv3.3 (*Kcnc3*) (Shakkottai et al. 2011; Bushart et al. 2021b) (Fig. 2a). BK (*KCNMA1*) is transcriptionally repressed in SCA1, but the mechanisms driving *Kcna6* and *Kcnc3* dysfunction in SCA3 are less clear. Intraventricular delivery of an ASO against the *ATXN3* gene in SCA3 transgenic mice restored proper Purkinje excitability and recovered expression levels of *Kcna6* and *Kcnc3* (Bushart et al. 2021b), suggesting that regulation of channel expression lies downstream of ATXN3 function and is perturbed by the polyglutamine expansion.

Although the majority of ion channel dysfunction in SCAs has focused on Purkinje neurons since they may be the most affected cell type (Kasumu and Bezprozvanny 2012), there is variability among SCAs in which cell populations are affected within the cerebellum. While mouse models of SCAs 1, 2, and 7 show irregular and slow PC spiking, underscoring the selective vulnerability of PCs in these CAG/polyglutamine repeat SCAs, SCA3 mouse models show irregular spiking not only in PCs (Shakkottai et al. 2011) but also in neurons of the deep cerebellar nuclei (DCN) (Mayoral-Palarz et al. 2022) (Fig. 2b). Interestingly, while irregular spiking in PCs and DCN neurons correlates with motor symptom onset, there is no worsening of this electrophysiological perturbation as clinical ataxia symptoms progress in a mouse model of the disease (Bushart et al. 2021a, b; Mayoral-Palarz et al. 2022). This lack of correlation suggests that the progressive ataxia seen in SCA3 is due, at least in part, to dysfunction outside the cerebellum. While neuropathological and imaging studies have confirmed atrophy of the brainstem, cerebral cortex, thalamus, and basal ganglia in SCA3 patients (Rüb et al. 2008; Rezende et al. 2018), the degree of electrophysiological perturbations in these regions remains unknown.

Many questions remain unanswered about the range and relevance of electrophysiological perturbations in SCA3 and other SCAs. That said, targeting neuronal dysfunction in the SCAs through specific ion channel modulation is a viable therapeutic strategy that should be pursued both as independent symptomatic and disease-modifying therapy, and as a complement to approaches aimed at the root genetic cause and other biochemical pathways.

More globally, addressing neuronal circuitry is important to consider for other SCAs as we move beyond a biochemical view of disease to a more systems-wide view. Examining synaptic transmission and neurotransmitter levels may uncover additional targets for SCA3 and other SCAs. For example, glutamate receptor

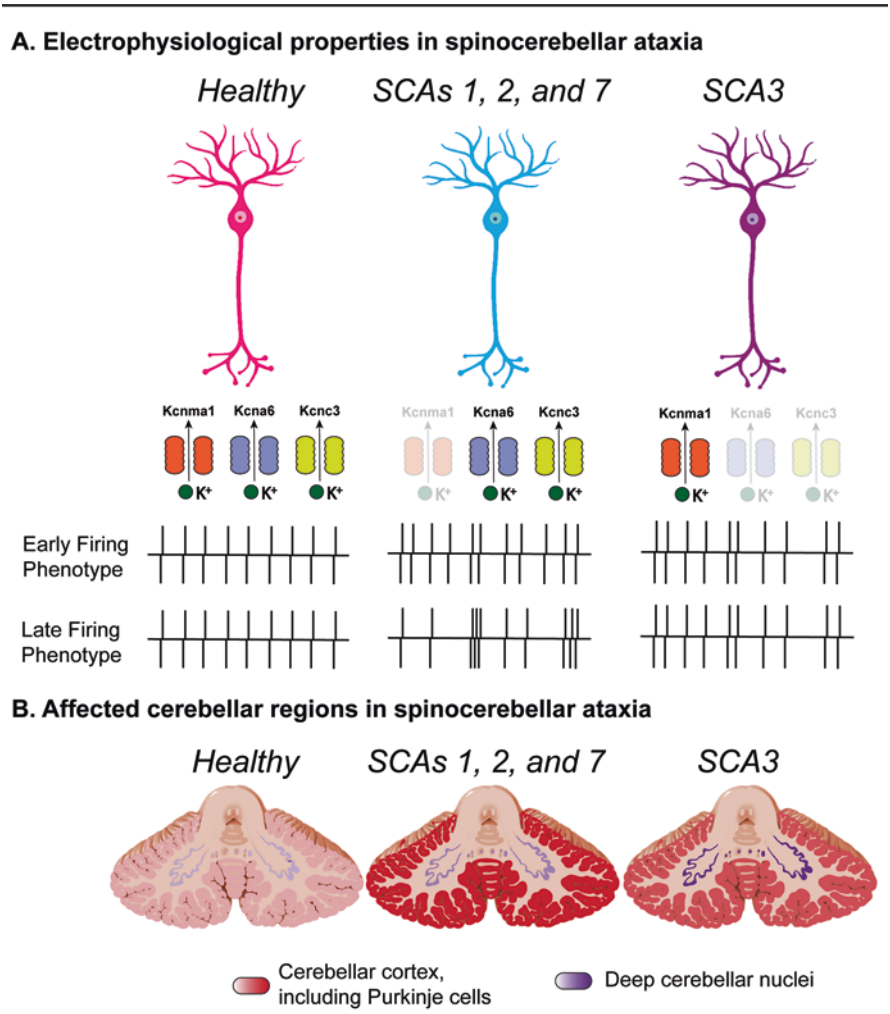

Fig. 2 Electrophysiological phenotyping in spinocerebellar ataxia. (**a**) The aberrant cerebellar spiking activity in SCAs can be traced to dysfunction or repressed expression of voltage-gated potassium channels. In SCAs 1, 2, and 7, decreased expression and function of *Kcnma1* (BK) seems to be the root driver of slow and irregular spiking and therefore represents an ideal target. In SCA3, however, *Kcnma1* expression remains unchanged while *Kcna6* and *Kcnc3* levels and activity are diminished. (**b**) Although the cerebellar cortex, and particularly the Purkinje cell layer, is predominantly affected in SCAs, SCA3 and several other SCAs can show marked involvement of the deep cerebellar nuclei. Gradient shading from lighter to darker indicates an increasing degree of involvement. (Image created with Biorender and Kenhub)

activity is perturbed in multiple SCAs (Meera et al. 2016, 2017) and is thought to lead to aberrant calcium signaling, potential depolarization block, and cytotoxicity. This mechanism has led to a Phase 3 Trial of troriluzole, a glutamate reuptake activator, the results of which are imminently pending.

6 Conclusion and the Future of SCA3 Therapeutics

The last few decades have seen an explosion in our understanding of the biological forces driving SCA3 pathogenesis. With this new knowledge and the recent advent of patient-derived biomarkers, the field is primed to move more therapeutics into the clinical phase than ever. For effective SCA3 therapeutics to advance to the next stage, we expect numerous leaps and shifts to occur. As mentioned above, patient-derived stem cell and organoid models will need to move to the main stage as platforms to understand disease biology and test novel therapeutics. The amenability of stem cells to genetic modification also opens the door to genetic therapies beyond ASOs including CRISPR-mediated correction of the CAG/polyglutamine expansion. Such efforts, already underway in cellular models, will prove challenging in patients. As CRISPR-based delivery and genetic manipulation techniques improve, however, we hope that such strategies will emerge as a viable option for patient intervention. While targeting the root cause of SCA3—the CAG/polyglutamine expansion—will likely serve as the base of many SCA3 therapeutic regimens, it is also clear that monotherapy may not be sufficient. Pharmacological agents focused on protein quality control machinery, bioenergetics, protein-protein interactions, and network connectivity will serve as valuable complements to further probe disease biology and improve patient outcomes.

Acknowledgments Our research and the preparation of this article are supported by NIH grants R25NS089450 (SS) R35NS122302 (HP), U01NS106670 (HP), and NIH T32 GM145470 (RP).

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Ion Channel Genes and Ataxia

Mahesh Padmanaban and Christopher M. Gomez

Abstract In this review we will discuss ataxic disorders attributed to mutations in ion channel genes. Such disorders seem to preferentially effect cerebellar Purkinje cell function, but not exclusively so. Some mutations result in gating alterations, such as loss-of-function or gain-of-function of the channels and others have no effect on channel function and may lead simply to poor trafficking or genetic deletion of the channel. Phenotypic presentation is varied and mutations in ion channel genes lead to congenital, static or progressive cerebellar ataxia and other features, including extrapyramidal symptoms, pyramidal symptoms, neuropsychological disturbance, autonomic dysfunction, and seizures among many others. Unique mutations of the same gene can also lead to completely different manifestations and phenotypes. While better characterization of some of these disorders may ultimately allow us to tailor individual therapy toward the particular type of ion channel dysfunction, the task of restoring channel expression will require more substantial advances. Here we endeavor to summarize the various types of ataxia related to ion channel gene mutations, their clinical features, and current data or theories on etiology of dysfunction.

Keywords Episodic ataxia · Spinocerebellar ataxia · Congenital ataxia · Phenotypic variability · Loss of function · Gain of function

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_7

1 Introduction

Genetically mediated cerebellar ataxias are a large and clinically heterogeneous group of disorders with common clinical features of cerebellar dysfunction, nystagmus, dysarthria, limb incoordination, imbalance, and unsteady gait. Mutation in ion channel genes causing ataxia is a subset or important subdivision of genetically mediated ataxias. Ion channels are pore-forming, water-filled macromolecular proteins that allow for diffusion of ions down an electrochemical gradient in a highly selective and efficient manner (Alexander et al. 2011; Liu and Wang 2019). They are key in processes such as neurotransmitter release, generating resting membrane potential and involved in each phase of the action potential. They are located in the plasma membranes or membranes of intracellular organelles (Alexander et al. 2011). Ion channels are critical for a variety of physiological and pharmacological functions. They can be classified by their gating mechanism, ion selectivity, and sequence homology (Liu and Wang 2019). Ion channels may be gated by changes in membrane voltage (voltage-gated), binding of ligands, such as second messengers or other intracellular/extracellular mediators (ligand-gated), and mechanosensitive mechanisms (stretch-gated) (Alexander et al. 2011; Liu and Wang 2019). Ion channels are prevalent with over 400 genes encoding them in the human genome (Alexander et al. 2011; Liu and Wang 2019). Thus, they are critical for a wide variety of physiological and pharmacological functions (Liu and Wang 2019). Alteration in ion-channel expression and function have the potential to greatly impact any cell type, especially neurons, which rely heavily on a wide array of ion channels, not only for excitability and signaling, but for normal development. Cerebellar ataxia is primarily associated with dysfunction and degeneration of cerebellar neurons, especially Purkinje cells, and mutations in numerous ion channel genes can lead to disrupted firing and/or affect viability, ultimately resulting in ataxia (Bushart and Shakkottai 2019).

In this review we will discuss the numerous ataxic disorders attributed to mutations in ion channel genes. We avoid the term "ion channelopathies," which has the connotation that all of these disorders result from an alteration in channel gating and, to some extent, result in an episodic presentation of symptoms. While some mutations in these disorders do result in gating alterations, such as loss-of-function or gain-of-function of the channels, and some disorders may even have an episodic presentation, this simplified view may result in unwarranted expectations of the promise of symptomatic alleviation from ion channel active drugs. In other cases, the mutations in the ion channel genes have no effect on channel function and may lead simply to poor trafficking or genetic deletion of the channel. Moreover, many mutations in ion channel genes lead to congenital, static or progressive cerebellar ataxia and other features, including extrapyramidal symptoms, pyramidal symptoms, neuropsychological disturbance, autonomic dysfunction, and seizures among many others. Interestingly, unique mutations of the same gene can also lead to completely different manifestations and phenotypes. While better characterization of some of these disorders may ultimately allow us to tailor individual therapy toward

the particular type of ion channel dysfunction, the task of restoring channel expression will require more substantial advances.

For more information on specific mutations related to the following conditions, please refer to the many excellent reviews referenced in this paper (Hasan and D'Adamo 2018; Döring et al. 2021; Park et al. 2019; Zhang et al. 2016; Pollini et al. 2020; Morin et al. 2020; Bailey et al. 2019; Casey and Gomez 2019; Casas-Alba et al. 2021; Coutelier et al. 2015; Storey 2014; Casey et al. 2017; Zambonin et al. 2017; Stendel et al. 2019; Schwarz et al. 2019; Gardella and Møller 2019; Nanetti et al. 2019).

2 Cerebellar Circuitry and Importance of Ion Channels

The cerebellum is composed of the cortex and deep cerebellar nuclei (DCN). The cortex is a trilaminar structure composed of the molecular layer, Purkinje cell (PC) layer, and granular layer (Kano and Watanabe 2020). Purkinje cells are the sole output neurons of the cerebellar cortex and their somata are aligned in the PC layer (Kano and Watanabe 2020). Purkinje cell dendrites extend into the molecular layer and project GABA-ergic (γ-aminobutyric acid) axons to the DCN and vestibular nuclei. Afferent fibers with excitatory input to Purkinje cells include mossy fibers, which originate in various extracerebellar regions, such as the spinal cord, pontine nuclei, and reticular formation, and climbing fibers, which originate in the inferior olive of the contralateral medulla oblongata (Palay and Chan-Palay 1974; Eccles et al. 1966). Climbing fibers cause strong depolarization of Purkinje cell dendrites, generating "complex spikes" in the soma consisting of fast somatic action potentials followed by slow calcium spikes due to activation of voltage-dependent calcium channels in Purkinje cell dendrites (Miyakawa et al. 1992; Eccles et al. 1966). Complex spikes consist of an initial spike immediately followed by a series of small spike oscillations superimposed on a sustained depolarization (Eccles et al. 1966). In contrast, mossy fibers convey motor and sensory information to the distal dendritic compartment of Purkinje cells through parallel fibers, the bifurcated axons of granule cells (Ito and Itō 1984). Each parallel fiber forms one, or occasionally two, synapses onto individual Purkinje cells and excitation results in a weak depolarization that would require input from many firing synchronously in order to generate a single action potential or "simple spike" similar to other neurons (Napper and Harvey 1988; Barbour 1993). Parallel fibers also excite two types of GABAergic interneurons, basket cells and stellate cells, which are contained in the molecular layer (Kano and Watanabe 2020). Basket cells interact with the soma and their axons surround the axon initial segment (AIS) of the Purkinje cell, while stellate cells interact with their dendrites (Palay and Chan-Palay 1974). These inputs modulate Purkinje cell intrinsic firing. The Purkinje cells ultimately contribute inhibitory input to neurons in the DCN and, in turn, the DCN neurons project to structures outside of the cerebellum, such as the red nucleus, thalamus and inferior olive,

modulating downstream motor pathways and influencing motor planning, execution and coordination (Kano and Watanabe 2020).

Purkinje cells exhibit pacemaking capability and can fire action potentials spontaneously without synaptic activation. One unique property that contributes to this feature is that Purkinje cells exhibit bistability of membrane potential, which is the property of having two distinct values at which the membrane potential is stable (Hoxha et al. 2018). At the more depolarized potential they generate tonic simple spike firing, while at the more hyperpolarized membrane potential they are silent (Hoxha et al. 2018). In vivo, Purkinje cell firing is irregular because it is shaped by incoming signals from parallel fibers, climbing fibers, and GABAergic interneurons as mentioned above. Another unique feature of Purkinje cells is that they are capable of high frequency discharge. Both the pacemaking and high-frequency discharge capabilities result from the unique ion channel makeup of the Purkinje cell.

In Purkinje cells, voltage-gated sodium channels, including Nav1.1 and Nav1.6, are important for action potential initiation, setting the threshold and propagation of action potentials. They also have a role in generating persistent current and give rise to a resurgent current during the repolarization phase, mainly produced through their interaction with the auxiliary Navβ4 subunit (Raman and Bean 1997; Ransdell et al. 2017; Grieco et al. 2005). These actions combine to confer spontaneous activity to Purkinje cells (Hoxha et al. 2018). Sodium currents must interact with potassium currents that are fast activating and deactivating in order to generate high firing frequencies and the Kv3 subfamily of high-voltage-activated potassium channels, 3 of which are expressed in Purkinje cells (Kv3.1, Kv3.3, Kv3.4), possesses these qualities (Hoxha et al. 2018). This quality is important because it allows faster repolarization and generation of after-hyperpolarization (Hoxha et al. 2018). Kv1 voltage-gated channels and Kv4 channels are subthreshold potassium channels. Kv1 channels are located at the axon initial segment, juxtaparanodal sites, and at the synaptic terminals and they are vital in axonal membrane repolarization after an action potential, adjusting the resting membrane potential and controlling neurotransmitter release (Hille 2001; Jan and Jan 2012). Kv1.1 and Kv1.2 are expressed in the terminals of basket cells and Kv1.2 is located in the dendrites of Purkinje cells, so they mainly affect GABAergic tone exerted by the inhibitory interneurons on Purkinje cells (Khavandgar et al. 2005). The Kv4.3 channel is the major component of subthreshold inactivating potassium currents in Purkinje cells and it is localized in Purkinje cell dendrites in association with Cav3 channels (Sacco and Tempia 2002; Hourez et al. 2011; Anderson et al. 2013). Voltage-gated calcium channels are responsible for calcium influx into the cell upon depolarization. Cav2.1 (P/Q type channel) and Cav3 family members (T-type), which are concentrated in the dendrites of Purkinje cells, are two such channels. Cav2.1 is co-localized with large-conductance voltage- and calcium-activated BK (Big K+) channels as well as small conductance calcium-dependent potassium channels, so that the net effect of calcium entry is an outward potassium current, which in turn hyperpolarizes the membrane potential (Bushart and Shakkottai 2019). This after-hyperpolarization (AHP) is essential for deactivation of voltage-gated sodium and potassium channels, which allows for their activation during subsequent action potentials (Bushart and

Shakkottai 2019). Other channels such as TRPC3 and the inositol 1,4,5-trisphosphate receptor play important roles mediating calcium homeostasis as well (Bushart and Shakkottai 2019).

3 Ataxia Related to Mutations in Potassium Channel Genes

3.1 Ataxia Related to Voltage-Gated Potassium Channels

Voltage-gated potassium channels play an essential role in action potential generation and propagation (Table 1). They are critical for setting the resting potential and degree of excitability of the membrane of the cell and influence the repolarization phase as well as action potential waveforms and firing patterns modulating synaptic activity. The voltage-gated potassium channels contain four subunits forming the central pore and each subunit contains six transmembrane domains (S1–S6), with S1–S4 forming the voltage sensor domain (VSD) and S5-S6 corresponding to the pore forming domain (Kuang et al. 2015) (Fig. 1). The VSD senses membrane potential alteration and undergoes conformational changes that are coupled to gate the pore forming domain (Kuang et al. 2015). The voltage-sensing region is in S4 and contains positively charged amino acids separated by hydrophobic residues making them electrically sensitive (Kuang et al. 2015; Suppiramaniam et al. 2010). The pore-forming subunits are most often comprised of α subunits (Suppiramaniam et al. 2010). Auxiliary subunits, sometime referred to as β subunits, are proteins which associate with α subunits and modulate the activity of K_V channels (Suppiramaniam et al. 2010). The genes encoding these different K channels have their own naming convention, each beginning with "*KCN*".

3.2 Kv1-Related Ataxia

The Kv1 family has eight members that are all expressed in the CNS (Masnada et al. 2017). The pore-forming α-subunits of these channels confer the Kv1 subtype and the genes encoding them are named with the convention, *KCNAx*, where "x" refers to the Kv1.x subfamily member (i.e., *KCNA1* encodes the Kv1.1 channel). Kv1 family channels are members of the delayed rectifier potassium channel family and are expressed predominantly in the axons and presynaptic terminals of the central nervous system (Döring et al. 2021). Kv1 α-subunits form tetrameric structures composed of four monomers or co-assemble with α-subunits of other members of the Kv1 family to form heterotetrameric channels with properties different from homomeric channels, including different kinetics and voltage dependence of channel gating (Vacher et al. 2008; Gutman et al. 2005). They possess slow inactivation (C-type or P-type) as well as fast type inactivation (N-type) caused by a "ball and

Table 1 Ataxia related to mutations in potassium channel genes

Ion channel name/gene	Ion channel type	Ataxia type	Mode of inheritance/ effect of mutation	Age of onset	Additional distinguishing features	Cerebellar atrophy present or not
Kv1.1/ KCNA1	Voltage-gated	Episodic ataxia type 1	Autosomal dominant/loss-of-function	First or second decade	Duration of attacks: seconds to minutes Interictal myokymia	Usually not, rarely atrophic
Kv1.2/ KCNA2	Voltage-gated		Autosomal dominant/loss-of-function, gain-of-function, mixed	Childhood onset (variable age)	Most common manifestation is early-onset developmental and epileptic encephalopathy with varying degrees of ataxia Seizures (90%), ataxia (64%), intellectual disability HSP phenotype	Present in 31% per one study
Kv3.1/ KCNC1	Voltage-gated	Myoclonic epilepsy and ataxia due to KCNC1	Autosomal dominant/loss-of-function	Childhood onset (mean age of 10)	Seizures, myoclonus; progression with stabilization in adulthood Normal development with gradual decline	Present in most cases
Kv3.3/ KCNC3	Voltage-gated	Spinocerebellar ataxia type 13	Autosomal dominant/loss-of-function or gain-of-function	Infantile onset Progressive childhood-onset Adult-onset	Infantile: delayed motor milestones, mild to moderate language impairment, and non-progressive with gradual lifetime improvement Childhood: mild to moderate cognitive impairment, mild motor delay, slowly progressive Adult: mild cognitive impairment, slowly progressive	Present

Kv4.3/ KCND3	Voltage-gated	Spinocerebellar ataxia type 19/22	Autosomal dominant/loss-of-function	Early onset Late onset	Early onset: Neurodevelopmental disorder, epilepsy are initial features; variable progression Late onset: Ataxia can be initial symptom; cognitive impairment, extrapyramidal features, slowly progressive	Present
Kir4.1/ KCNJ10	Voltage-gated	SeSAME syndrome/ EAST syndrome	Autosomal recessive/loss-of-function	Infancy to childhood	Seizures, sensorineural deafness, neurodevelopmental disorders, nephropathy; may be nonprogressive	Present
BK/ KCNMA1	Calcium- and voltage-gated		Autosomal dominant/loss-of-function	Congenital to infancy	Intellectual disability, seizures, dyskinesia, dystonia Can be progressive	May be present

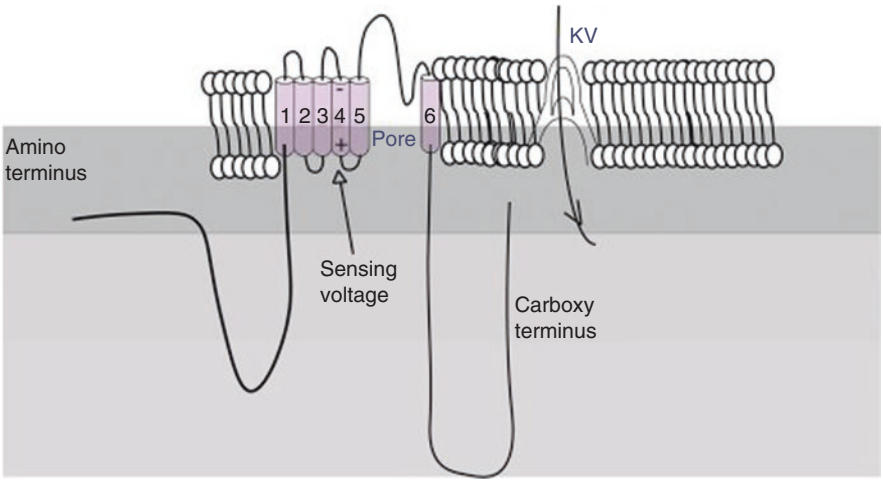

Fig. 1 Voltage-gated potassium channel structure (Mohammad 2020). Channel consists of transmembrane segments S1–S6. The S4 segment functions as a voltage sensor that triggers opening of the channel by undergoing a conformation change upon membrane depolarization, while S5–S6 form the ion-conducting pore with its selectivity filter localized in the pore loop (Kuang et al. 2015; Imbrici et al. 2021)

chain" mechanism with pore occlusion (Hasan and D'Adamo 2018). Four β subunits participate in the ion channel complex and provide four inactivation particles for this very process (Hasan and D'Adamo 2018).

3.2.1 KCNA1-Related Ataxia/Episodic Ataxia Type 1 (EA1)

EA1 is characterized by intermittent episodes of generalized ataxia, loss of balance, incoordination of the hands, tremor, dysarthria, muscle twitching/stiffening, vertigo, diplopia, dysarthria, dyspnea, nausea, headache, and diaphoresis (Hasan and D'Adamo 2018; Hasan et al. 2017; D'Adamo et al. 2015). Less common symptoms include choreoathetosis, carpal spasm, neuromyotonia, nystagmus, and hyper- or hypothermia (Hasan et al. 2017; Hasan and D'Adamo 2018; D'Adamo et al. 2015). Symptoms typically manifest in the first or second decade of life and the attacks can last seconds to minutes, but longer attacks lasting hours have been described (Hasan and D'Adamo 2018). Frequency of attacks is variable. Triggers for an attack include stress or anxiety, intercurrent illness or fever, excitement or emotional upset, fatigue, menstruation or pregnancy, environmental temperature, including hot baths or use of a hairdryer, startle response, abrupt movements or sudden postural changes (kinesiogenic stimulation), vestibular stimulation, exercise, and ingestion of caffeine, alcohol, excessive salt, bitter oranges, and chocolate (Imbrici et al. 2008; Hasan and D'Adamo 2018). Interictal ataxia has not been reported in EA1, but interictal myokymia is a feature (Imbrici et al. 2008; Hasan and D'Adamo 2018).

Other interictal features may include cognitive dysfunction, increased muscle tone, and seizures have also been reported (Hasan and D'Adamo 2018). MRI brain is usually normal, and only rarely cerebellar atrophy is seen (Hasan and D'Adamo 2018).

EA1 is an autosomal dominant condition with incomplete penetrance caused by mutations in the *KCNA1* gene on chromosome 12p13 (D'Adamo et al. 2015). Most individuals have an affected parent, but de novo mutations also occur. *KCNA1* encodes the voltage-gated potassium channel Kv1.1, which is the α-subunit of the voltage-gated delayed-rectifier potassium channel (Hasan and D'Adamo 2018). Kv1.1 is mostly found co-assembled with Kv1.2 subunits and this heterotetramer is expressed highly in cerebellar basket cell terminals and at the juxtaparanodal region of motor axons (Hasan and D'Adamo 2018). Kv1.1 channels regulate neuromuscular transmission, control the release of GABA from cerebellar basket cells onto Purkinje cells, and are known for their role in controlling the excitability of cerebellar, hippocampal, cortical, and peripheral nervous system neurons (Hasan and D'Adamo 2018; D'Adamo et al. 2015). Pathogenic missense variants of *KCNA1* are most common, but nonsense variants and deletions are also reported, resulting in loss-of-function and reduced efflux of potassium with reduction in the outward current (Hasan and D'Adamo 2018). Several factors are thought to contribute to this including:

1. Altering voltage dependence and changing the channel gating kinetics affecting opening and closing.
2. Altered assembly or incorporation into the tetramers that form the channels and leading to a dominant negative effect when combined with wild-type protein.
3. Altered trafficking and reducing the amount of Kv1.1 in the plasma membrane (D'Adamo et al. 2015; Hasan et al. 2017).

The reduced delayed rectifier function of Kv1.1 in the cerebellar basket cells may increase their membrane excitability, prolong their action potential duration, and enhance Ca2+ ion influx (D'Adamo et al. 2015; Hasan and D'Adamo 2018). They would then release larger amounts of GABA reducing the inhibitory output of the Purkinje cells and ultimately altering cerebellar output (D'Adamo et al. 2015; Hasan and D'Adamo 2018).

3.2.2 KCNA2-Related Ataxia

KCNA2 mutations cause a wide variety of phenotypes and symptoms, including early-onset developmental and epileptic encephalopathy, milder forms of epilepsy, intellectual disabilities, cerebellar dysfunction including episodic ataxia, and even hereditary spastic paraplegia (HSP) (Döring et al. 2021; Corbett et al. 2016; Helbig et al. 2016). The most prevalent manifestation has been early-onset developmental and epileptic encephalopathy with varying degrees of ataxia (Döring et al. 2021). Seizures were present in 90% of patients and ataxia in 64% of reported cases with gait, dysarthria, impaired coordination, hypotonia, and tremor being the most common features (Masnada et al. 2017; Döring et al. 2021). The HSP phenotype is one of

the few mutations that has reported seizure-free cases (Helbig et al. 2016; Manole et al. 2017). Most individuals had some degree of intellectual disability (Döring et al. 2021). Behavioral features and cranial dysmorphism were rarely associated features (Masnada et al. 2017). MRI brain revealed cerebellar atrophy in 31% of cases in which it was available (Masnada et al. 2017; Döring et al. 2021). Döring et al. (2021) postulated that a clinical spectrum exists and follows a pattern according to the eventual outcome of the mutation on overall channel function. The subgroups differ in regard to age at seizure onset, development before onset of seizures, outcome of seizures, and intellectual disability (Döring et al. 2021). They found that patients with a pathogenic variant conferring combined loss-of-function and gain-of-function effects had more severe phenotypes overall showing early developmental abnormalities and earlier epilepsy onset compared with patients whose mutations conferred either loss-of-function or gain-of-function alone, but all three could lead to epileptic encephalopathy (Döring et al. 2021). In addition to general trends, loss-of-function variants showed better development prior to seizure onset, later onset of epilepsy, more favorable outcomes with epilepsy, and less severe intellectual disability when compared to mutations that caused mixed dysfunction (Döring et al. 2021). Gain-of-function variants have significantly later onset of epilepsy compared to mixed dysfunction variants, but they do not differ significantly in development before seizures, epilepsy outcome, and intellectual disability (Döring et al. 2021). Head-to-head comparison of gain-of-function to loss-of-function variants did not yield significant differences in these categories. Ataxia was present in all cases reported with gain-of-function and mixed cases and the majority of patients with loss-of-function variants (Döring et al. 2021). In addition, some recurrent variants also lead to variant-specific characteristics, such as the one causing an HSP phenotype.

The *KCNA2* gene encodes the voltage-gated potassium channel subfamily Kv1.2, which is a low voltage, slow inactivating channel that opens with small depolarizations close to the resting potential (Imbrici et al. 2021). Of the 30 reported autosomal dominant pathological variants affecting *KCNA2*, the majority of variants were de novo, but a fair number were familial (Döring et al. 2021). Twenty-six of these were missense, one was an in-frame deletion, and three lead to truncation (Döring et al. 2021). While missense mutations could lead to loss-of-function, gain-of-function, or mixed dysfunctionality, the other types all lead to loss-of-function (Döring et al. 2021). The mutations tended to occur in highly conserved areas in the N-terminus, S3 segment, voltage sensing S4 segment, S5/S6 segment, and pore regions (Masnada et al. 2017). Electrophysiological characterization has been achieved in 43% of the known variants involving the *KCNA2* gene. Six were associated with loss-of-function, three with gain-of-function, and four with combined effects (Döring et al. 2021). Gain-of-function mutations are thought to hyperpolarize the membrane potential and slow channel closure, thus inhibiting the firing of neurons expressing such mutations (Masnada et al. 2017; Imbrici et al. 2021). Loss-of-function mutations are thought to impair repolarization during an action potential and dampen excitability (Masnada et al. 2017). Inhibitory interneurons and Purkinje cells harboring these mutations may then contribute to seizures and ataxia, respectively. These mutations could also affect the makeup of the heterometric channels

and cause a dominant negative effect and lead to impaired trafficking to appropriate destination in plasma cell membrane (Masnada et al. 2017). The reasons for the more severe presentation in mutations that cause mixed dysfunctionality are not completely understood, but one theory is that the mutations have different effects depending on the cell type (Döring et al. 2021). In other words, different neuronal subpopulations may be impaired in different ways (either loss or gain-of-function) and lead to the more severe phenotype. For instance, GABA-ergic interneurons may be impaired in addition to dysfunction in glutamatergic excitatory neurons leading to different effects on different networks that combine to create the more severe phenotype (Döring et al. 2021). A loss-of-function mutation conferred on Xenopus laevis oocytes was shown to lead to proton currents in addition to loss of Kv1.2 function (Starace and Bezanilla 2004; Helbig et al. 2016). A loss of potassium currents combined with gain-of-function from leaky proton currents could then explain why such mutations lead to seizures (Döring et al. 2021). Also, as alluded to above, certain recurrent variants also are associated with certain phenotypic features. The variant p.(Arg294His) is associated HSP and ataxia with seizures being described rarely (Helbig et al. 2016; Manole et al. 2017). The variant p.(Met255_Ile257del) is associated with episodic ataxia, normal intellectual abilities, and self-limited epilepsy (Corbett et al. 2016).

3.3 Kv3-Related Ataxia

The Kv3 subfamily consists of subunits Kv3.1–Kv3.4 that are encoded by four genes, *KCNC1–KCNC4* (Nascimento and Andrade 2016). Subunits Kv3.1–Kv3.4 assemble into homo- or heterotetramers to form the voltage-gated channel (Nascimento and Andrade 2016). The channel is a fast activating/deactivating channel, so it produces rapid repolarization with little to no refractory period and reduces afterhyperpolarization allowing neurons expressing the channels to fire trains of action potentials at high frequencies (Zhang et al. 2016; Munch et al. 2018). Therefore, these channels tend to be expressed in neurons that fire at high rates such as in the brainstem, certain cortical regions and cerebellum (Bürk et al. 2013; Zhang et al. 2016). Purkinje cells are among the cells that express these channels, which likely explains why there is cerebellar manifestation and ataxia in such patients (Zhang et al. 2016). Mutations in two such channel genes, *KCNC1* and *KCNC3*, are associated with ataxia.

3.3.1 KCNC1-Related Ataxia/Myoclonic Epilepsy and Ataxia Due to KCNC1 (MEAK)

MEAK is a type of progressive myoclonic epilepsy, all of which are characterized by myoclonus, seizures, and gradual neurological decline (Muona et al. 2015). Disease onset is around a mean age of 10 with near normal development preceding

this, but the symptoms at onset can vary (Oliver et al. 2017). Myoclonus is the initial symptom for most patients and it progressively worsens in addition to being intermittently exacerbated by action, stress, and startle (Barot et al. 2020). Due to the degree of myoclonus, some are wheelchair bound by teen years (Oliver et al. 2017). The progression does stabilize in adulthood. Seizures have been reported as the initial symptom in a few patients (Barot et al. 2020). In a series of 20 patients, 19 had ataxia (Oliver et al. 2017). Importantly, the lack of hearing loss, retinal abnormalities, and sensory impairment helps to set it apart from other progressive myoclonic epilepsies (Barot et al. 2020). Cognitively, a low normal range of verbal performance and overall mild decline can be seen (Barot et al. 2020). Brain MRI may show global symmetrical cerebellar atrophy of a moderate degree in most cases but can be normal and can also stabilize once patients reach adulthood (Barot et al. 2020; Nascimento and Andrade 2016; Oliver et al. 2017). Thickening of the corpus callosum has also been observed (Oliver et al. 2017).

The causative gene is the *KCNC1* gene located on chromosome 11p15.1 and the condition is autosomal dominant (Barot et al. 2020). *KCNC1* encodes Kv3.1 (Muona et al. 2015). A common heterozygous missense mutation resulting in the substitution of histidine for arginine at codon 320 (p.Arg320His) is the cause of MEAK (Muona et al. 2015). The mutation is generally de novo, but familial cases have been reported and can be associated with parental mosaicism (Kim et al. 2018). The mutation targets arginine contained in the highly conserved S4 voltage sensor region of the channel (Nascimento and Andrade 2016). Mutant channels hardly produced a current when the membrane was depolarized in one study, suggesting loss-of-function, but there was also potentially a dominant-negative effect exhibited by the fact that a decrease in current was observed even when co-expressed with the wild type protein in heterotetramers (Nascimento and Andrade 2016). One study revealed a hyperpolarizing shift in the voltage dependence of activation compared to the wild-type protein and slowing of channel activation and inactivation (Munch et al. 2018). Yet another potential mechanism of dysfunction revealed was the reduction in expression of Kv3.1 at the plasma membrane suggesting mis-localization may play a part as well (Munch et al. 2018). The decreased potassium current disrupts firing properties of fast-spiking neurons, affecting neurotransmitter release and inducing cell death (Nascimento and Andrade 2016). This malfunction is thought to mostly affect inhibitory GABAergic interneurons, leading to myoclonus and seizures due to hyperexcitability, and cerebellar neurons, leading to ataxia (Nascimento and Andrade 2016; Oliver et al. 2017).

3.3.2 KCNC3-Related Ataxia/Spinocerebellar Ataxia Type 13 (SCA13)

SCA13 is a rare disorder with a phenotypic spectrum that includes infantile-onset, progressive childhood-onset, and adult-onset cerebellar ataxia (Waters 2020). The infantile-onset form is non-progressive and characterized by limb, truncal, and gait ataxia, dysarthria, tremor, delayed motor milestones, mild to moderate language impairment, and gradual lifetime improvement of both motor and cognitive

symptoms (Minassian et al. 2012; Waters 2020). Nystagmus, hyperreflexia, psychiatric manifestations, myoclonus, and seizures may also occur (Waters 2020). Cerebellar hypoplasia is seen on MRI (Khare et al. 2017). Childhood-onset SCA13 is characterized by slowly progressive ataxia with gait ataxia, dysarthria, mild to moderate cognitive impairment, and mild motor delays (Waters 2020). Nystagmus, seizures and pyramidal signs were also observed in some individuals (Waters 2020). Slowly progressive atrophy of the cerebellum is seen on MRI with this subtype as well (Waters 2020). Adult-onset SCA13 is also slowly progressive and characterized by gait, truncal and appendicular ataxia, titubation, dysarthria, hypotonia, and mild cognitive impairment (Subramony et al. 2012; Waters 2020). Oculomotor signs are not common (Waters 2020). Myoclonus has been described in some cases (Montaut et al. 2017; Subramony et al. 2013). Sound localization due to insensitivity to changes in the amplitude or timing of sounds arriving at the two ears is also seen in such individuals (Zhang et al. 2016). Spasticity has also been documented (Khare et al. 2018). While MRI findings include mild to moderate cerebellar atrophy, predominantly in the midline, the atrophy can precede clinical symptoms (Waters 2020). Cortical atrophy may also be seen (Waters 2020). None of the three subtypes appear to affect life expectancy (Waters 2020).

SCA13 is an autosomal dominantly inherited condition and is fully penetrant with unknown prevalence (Waters 2020). De novo mutations are rare. The affected gene is the *KCNC3* gene on chromosome 19q13.33 that encodes the voltage-gated potassium channel Kv3.3 (Bürk et al. 2013; Zhang et al. 2016). Proposed mechanisms include dominant-negative effect or gain-of-function. Certain pathogenic variants are associated with different phenotypes. These include the p.Arg423His variant, which has been associated with congenital-onset, the p.Phe448Leu and the p.Val535Met variants, which are associated with childhood-onset, and the p.Arg420His and p.Pro583_Pro585del variants, which are associated with adult-onset SCA13 (Waters et al. 2006; Minassian et al. 2012; Bürk et al. 2013; Duarri et al. 2015; Montaut et al. 2017; Khare et al. 2018). Some of these variants, such as p.Arg420His, result in a nonfunctional protein that combines with the wild-type protein in the heterotetramers or does not insert into the plasma membrane due to degradation or sequestration in the cell elsewhere, exerting a dominant-negative effect or loss-of-function and lower current amplitude (Zhang et al. 2016). In contrast, other variants, such as p.Phe448Leu, result in a fully functional protein incorporated into the channel, but the voltage-dependent activation is shifted toward a more negative potential, slowing the rate of channel closure following deactivation, resulting in a prolonged open state of the channel (Zhang et al. 2016; Waters et al. 2006). The effects of both changes would be an increase in the amount of potassium current evoked by physiological depolarizations (Zhang et al. 2016). Alterations in gating or changes in kinetic behavior and voltage dependence of the channel are thought to be associated with earlier onset of the disease (Zhang et al. 2016; Minassian et al. 2012). The mechanisms leading to cerebellar degeneration in SCA13 are uncertain, but one hypothesis is that the abnormal accumulation of intracellular calcium due to longer duration spikes results in neurotoxicity and/or formation of inappropriate axonal or dendritic connections triggering cell death (Irie et al.

2014; Waters et al. 2006; Zhang et al. 2016). Another possible contributor to degeneration of cerebellar neurons, may be due to the association between Kv3.3 channels and the Hax-1protein (Zhang et al. 2016, 2021a, b). Kv3.3 binds and stimulates Tank Binding Kinase 1 (TBK1), an enzyme that controls trafficking of membrane proteins into multivesicular bodies and is also required for binding of Kv3.3 to its auxiliary subunit Hax-1 (Zhang et al. 2016, 2021a). Hax-1 prevents Kv3.3 channels from rapid inactivation during sustained depolarization and also functions as an anti-apoptotic protein required for survival of cerebellar neurons (Zhang et al. 2016, 2021a). Excessive activation of TBK1 by disease-causing Kv3.3 mutations leads to the loss of Hax-1 by its trafficking and accumulation in multivesicular bodies and lysosomes (Zhang et al. 2021a). Overactivation also results in exosome release from neurons, a process that leads to activation of caspases and increased cell death (Zhang et al. 2016, 2021a).

3.4 KCND3-Related Ataxia/Spinocerebellar Ataxia Type (SCA19/22)

SCA19 and SCA22 were identified in two separate families that seemed to have different phenotypes, but involved mutations in a common gene, *KCND3*. In subsequent years, the phenotypic spectrum has broadened and the condition is now often referred to as SCA19/22 as they are thought to refer to the same disease (Pollini et al. 2020; Huin et al. 2017). Similar to SCA13, SCA19/22 has a spectrum that includes an early onset form and a late onset form with a wide variety of associated features. A recent review of 68 cases, revealed that ataxia was the presenting sign in 42 with other presenting signs including epilepsy, neurodevelopmental disorder (developmental delay progressing to intellectual disability), episodic ataxia or other episodic neurological symptoms, head tremor, diplopia, or psychiatric symptoms (Pollini et al. 2020). Cerebellar features can include gait ataxia, imbalance, dysmetria, dysdiadochokinesia, intentional tremor, oculomotor disturbances (saccadic smooth pursuit, gaze-evoked-nystagmus most common; less frequently saccadic dysmetria and slow saccades), and dysarthria with a pure cerebellar syndrome detected in 22 patients with late onset (Pollini et al. 2020). Nearly all patients exhibited static or progressive ataxia (Pollini et al. 2020). Other features include non-cerebellar related oculomotor disturbances (vertical ophthalmoplegia and supranuclear gaze palsy), cognitive impairment of varying degrees (mostly visuospatial and executive function), extrapyramidal symptoms (including parkinsonism most frequently, but also dystonia, myoclonus, and tremor), epilepsy (nearly 50% of early onset), pyramidal symptoms (hyperreflexia, spasticity), psychiatric symptoms (depression, anxiety, obsessive compulsive disorder, delusional thoughts) and peripheral neuropathy (late onset only) (Pollini et al. 2020). Brain MRI revealed atrophy of the vermis or cerebellar hemispheres, or global cerebellar atrophy (Pollini et al. 2020). The early onset form typically presents with some form of a

neurodevelopmental disorder (developmental delay, intellectual disability, learning disability) or epilepsy prior to the onset of cerebellar signs, but can present with cerebellar manifestation as well (Pollini et al. 2020). The rate of progression of ataxia, onset and severity are less predictable in early onset SCA19/22. Ataxia was the most frequent presentation of late-onset SCA19/22. Late-onset patients were also more likely to exhibit a phenotype of pure cerebellar ataxia, but cognitive decline, peripheral neuropathy, and extrapyramidal symptoms coexisted as well (Pollini et al. 2020). Ataxia is often slowly progressive with patients bedridden or in a wheelchair three to five decades after onset (Pollini et al. 2020). Cognitive impairment was often mild and present in 25% of the patients with late onset, mostly affecting executive and visuospatial function. A unique presentation for some individuals was one of paroxysmal symptoms, including ataxia and other neurological manifestations (Pollini et al. 2020). However, it is unclear how they progressed as they have not been longitudinally followed (Pollini et al. 2020).

The causative gene of SCA19/22 is *KCND3* of chromosome 1p21q23 (Pollini et al. 2020). The condition is autosomal dominant and both missense mutations and deletions have been identified. *KCND3* encodes the voltage-gated potassium channel Kv4.3, which is highly expressed in the central nervous system, particularly in cerebellar Purkinje cells, deep nuclei, granule cells, and interneurons (Kollo et al. 2006). The channel may also play a role in migration and cerebellar development as Purkinje cells express different levels during the states of migration (Pollini et al. 2020). A common region for mutation is between the pore loop and segment 5 and 6 directly affecting the channel pore, but other locations have been found as well (Pollini et al. 2020). *KCND3* mutations are thought to result in loss-of-function when presenting with a neurological manifestation, but there are rare exceptions (Pollini et al. 2020). Similar to SCA13 and KCNC3 mutations, potential mechanisms include a shift in channel gating to more depolarized voltages, a nonfunctional protein product, decreased expression due to impaired trafficking to membrane or degradation, each of which may contribute to reduction in outward potassium currents (Pollini et al. 2020; Zanni et al. 2021; Hsiao et al. 2019). A dominant negative effect of the gene is also thought to play a part in the pathogenesis, similar to other voltage-gated potassium channel disorders (Hsiao et al. 2019). Gain-of function mutations involving *KCND3* generally have been associated with cardiac manifestations, including Brugada's syndrome and early-onset atrial fibrillation (Pollini et al. 2020). The reason for this discrepancy is unclear.

3.5 KCNJ10-Related Ataxia/SeSAME Syndrome

SeSAME syndrome (Seizures, Sensorineural deafness, Ataxia, Mental retardation and Electrolyte imbalance) also known as EAST syndrome (Epilepsy, Ataxia, Sensorineural deafness, and Tubulopathy) is a rare cause of inherited ataxia with an estimated prevalence of 1:1,000,000 (Bockenhauer et al. 2009; Scholl et al. 2009; Suzumoto et al. 2021). The presenting symptom is often generalized tonic-clonic

seizures that are responsive to anti-epileptic therapy that occurs as early as infancy (Suzumoto et al. 2021). Ataxia and sensorineural hearing loss of variable severity usually manifest later in the disease course and may be non-progressive (Suzumoto et al. 2021; Celmina et al. 2019). Ataxia may be characterized by ataxic gait, intention tremor, dysmetria, dysdiadochokinesia, speech with cerebellar patterns, and titubation (Celmina et al. 2019). Neurodevelopmental delay, including delay in language and motor skills, can occur as well as cognitive impairment of varying degrees (Suzumoto et al. 2021). Renal involvement is characterized by salt-losing nephropathy with hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria resembling Gitelman Syndrome (Suzumoto et al. 2021). Spasticity and upper motor neuron signs have also been described (Morin et al. 2020). MRI brain has most commonly shown cerebellar atrophy, signal abnormalities in cerebellar structures, a thin corpus callosum, and brainstem hypoplasia (Celmina et al. 2019).

SeSAME syndrome is inherited in an autosomal recessive fashion and mutations are thought to result in loss-of-function (Suzumoto et al. 2021). The gene affected in SeSAME syndrome is the *KCNJ10* gene on chromosome 1q23.2, which encodes Kir4.1, a member of the inwardly rectifying potassium (Kir) channel family that are essential in control of resting membrane potential, coupling of the metabolic cellular state with membrane excitability, and maintenance of potassium homeostasis in diverse cell types. (Tang et al. 2010). In the brain, Kir4.1 is expressed predominantly in astrocytes and oligodendrocytes, where it accounts for extracellular potassium buffering, glutamate uptake, astrocyte development, and myelination (Neusch et al. 2001; Kucheryavykh et al. 2007; Djukic et al. 2007). Seizures are thought to occur due to decreased potassium clearance from the synaptic cleft after build up from the cycle of excitation and repolarization of neurons, which causes considerable sodium influx into neurons and potassium efflux (Abdelhadi et al. 2016). Glial cells are important in protective "spatial buffering," taking up excess potassium ions and distributing these ions through gap junctions (Abdelhadi et al. 2016). When glial cells are unable to perform this function, potassium released from neurons accumulates in the extracellular space, the membrane potential remains decreased and neurons are susceptible to further excitation and generation of seizures (Celmina et al. 2019; Abdelhadi et al. 2016). Kir4.1 is also highly expressed in the basolateral membrane of the distal nephron (the cortical thick ascending limb of Henle's loop, distal convoluted tubule, connecting tubule and cortical collecting duct) where it contributes to potassium recycling and generation of a negative membrane potential in addition to maintaining a stable source of extracellular potassium needed for the Na+/K+ ATP-ase driven transcellular sodium reabsorption (Suzumoto et al. 2021; Zhang et al. 2019; Celmina et al. 2019). In the inner ear, Kir4.1 regulates potassium homeostasis and contributes to the high potassium concentration in the endolymph that helps to generate the positive endocochlear potential, which is essential for cochlear development and hearing, and it is also important for spiral ganglion neuron excitation (Chen and Zhao 2014). The Kir4.1 protein is made up of two transmembrane helices and an extracellular loop, which folds back to form the lining of the pore and acts as an ion selectivity filter (Celmina et al. 2019). The Kir channels are typically composed of four subunits that are either homo- or heterotetramers

with homotetramers of Kir4.1 being present in the brain and inner ear and heterotetramers forming with Kir5.1 in the kidney (Celmina et al. 2019). While many different variants have been reported in association with SeSAME syndrome, most cases are the result of homozygous or compound heterozygous mutations, with other types of mutations (e.g., nonsense) rarely occurring (Zhang et al. 2019). One study comparing homozygous frameshift mutations with missense mutations found that frameshift mutations resulting in the truncation and loss of portions of the C-terminal region of Kir4.1 are thought to result in a more severe phenotype because they result in a nonfunctional protein (Suzumoto et al. 2021). Other mechanisms leading to loss-of-function may be mis-trafficking that may be due to alteration in interaction with anchor proteins, instability leading to degradation, and reduced permeability to potassium ions (Zhang et al. 2019; Suzumoto et al. 2021; Celmina et al. 2019). Phosphatidlyinositol 4,5 bisphosphate or PIP2 is a component of cell membranes that activates Kir channels and one mutation was thought to alter Kir4.1 in such a way to weaken the interaction with PIP2 leading to a decrease in channel activity (Zhang et al. 2019).

3.6 KCNMA1-Related Ataxia

A small number of patients with congenital ataxia and other neurological features have been described with mutations in the gene, *KCNMA1*, typically with resulting loss of function. Intellectual disability, seizures, dyskinesia, and dystonia were variably present in these patients as well (Bailey et al. 2019). Some of these patients also had cerebellar atrophy on MRI (Bailey et al. 2019). A case of progressive ataxia due to a de novo mutation in the gene *KCNMA1* was recently described in a 16-year-old patient with onset of symptoms around 18 months characterized by severe dysarthria, intention tremor, dysmetria, dysdiadochokinesia and wide-based ataxic gait and stance (Du et al. 2020). She also had coarse downbeat nystagmus, gaze evoked nystagmus in all directions, perioral, truncal and limb dyskinesia and spastic tone with bilateral Babinski signs (Du et al. 2020). She had a delay in motor skills with motor coordination initially improving, but still lagging behind her younger sibling and eventually worsening at age 8 (Du et al. 2020). She was developmentally delayed, had slight difficulty with comprehension and following conversations, and exhibited emotional outburst (Du et al. 2020). Serial MRI brain scans from age 7 to 15 exhibited severe, progressive cerebellar atrophy that was most prominent in midline.

The singular patient and the review of cases outlined above were found to have heterozygous de novo mutations by exome sequencing in the *KCNMA1* gene on chromosome 10q22.3 that encodes the BK channel α-subunit that leads to loss-of-function (Du et al. 2020; Bailey et al. 2019). The BK channel, which is widely expressed in the central nervous system and abundant in Purkinje cells, is a large conductance calcium- and voltage-activated potassium channel. It has a pore-forming α-subunit that contains seven transmembrane segments (S0–S6) with an

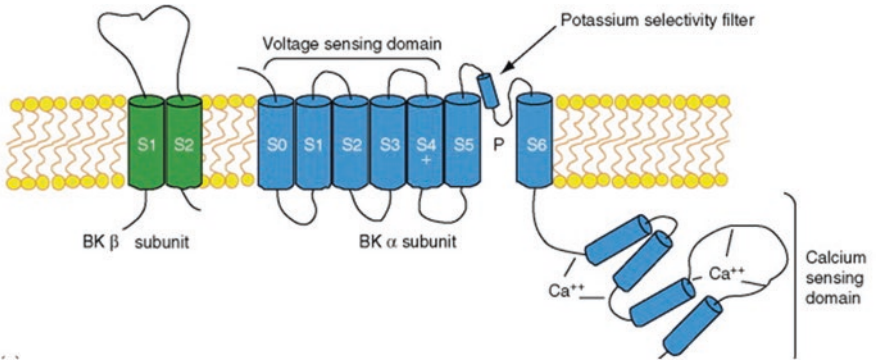

Fig. 2 BK channel structure (Wang et al. 2009). The channel consists of an α-subunit that contains transmembrane segments S0–S6 with an extracellular N terminus and an intracellular C terminus containing two high affinity calcium binding sites that mediate allosteric gating (Latorre et al. 2017). The S1–S4 segments contain positively charged residues and constitute the voltage sensor while S5–S6 form the pore, which is the location of a highly selective potassium conduction pathway or filter (Yang et al. 2015)

extracellular N terminus and an intracellular C terminus containing two high affinity calcium binding sites that mediate allosteric gating of BK channels (Latorre et al. 2017) (Fig. 2). The S1–S4 segments contain positively charged residues and constitute the voltage sensor while S5–S6 form the pore, which is the location of a highly selective potassium conduction pathway or filter (Yang et al. 2015). The channels are activated by both membrane depolarization and cytosolic calcium, and mediate potassium efflux leading to repolarization, hyperpolarization, afterhyperpolarization and decreased excitability. They play a role in quickly repolarizing the cell membrane when intracellular calcium is increased and in shaping the afterhyperpolarization potential (Womack et al. 2009). BK channels are also present in organelles including the inner mitochondrial and inner nuclear membranes (Li and Gao 2016). The de novo mutation in the singular patient predicted a G345S mutation in a highly conserved amino acid sequence among potassium selective channels contained within the selectivity filter (Du et al. 2020). Reduction in selectivity to potassium ions increases relative permeability to sodium ions (Du et al. 2020). BK channels normally damp excitatory processes mediated by an increase in internal calcium, so increased sodium permeability means more sodium movement inward and prolonged depolarization (Du et al. 2020). BK channels containing such a mutation exhibited dramatically reduced unitary conductance relative to wild-type channels and proportional reduction in macroscopic potassium currents was found to suggest loss-of-function (Du et al. 2020). They also suspected a profound dominant-negative effect on the channels if heterotetramers of wild type and mutant protein existed given those facts (Du et al. 2020). BK channels are also found on mitochondria and mutants led to depolarization and overall depletion of mitochondria (Du et al. 2020). Expression of the mutation led to reductions in neurite outgrowth, cellular viability, and depolarization and depletion of mitochondria (Du et al. 2020).

4 Ataxia Related to Mutations in Calcium Channel Genes

4.1 Ataxia Related to Voltage-Gated Calcium Channels

Voltage-gated calcium channels regulate calcium entry into cells especially neurons, which makes them important in regulating the membrane potential as well as in modulation of calcium signaling pathways including neurotransmitter release, neurite outgrowth, calcium-dependent gene transcription, or regulation of enzymes (Table 2). They form hetero-oligomeric complexes containing an α1 subunit with pore-forming domains and auxiliary subunits including β, g, and α2–δ (Escayg et al. 2000) (Fig. 3). The α1 subunit also provides the extracellular binding site for agonists and antagonists (Alexander et al. 2011). Each α1 subunit has four homologous repeats (I–IV), each repeat having six transmembrane domains (S1–S6) and a pore-forming region between transmembrane domains S5 and S6 (Alexander et al. 2011). Gating is thought to be associated with the membrane spanning S4 segment, which contains highly conserved positive arginine residues that are the voltage sensing elements. Mutation is expected to affect the voltage dependency of the channel by affecting opening of the pore (Alexander et al. 2011; Coutelier et al. 2015; Hashiguchi et al. 2019). At least for high-voltage activated channels, it is likely that native channels comprise co-assemblies of α1, β and α2–δ subunits (Alexander et al. 2011). The interaction of α1 with these other subunits influences the properties of the channel (Catterall 2011). As an example, the interaction with beta subunits modulates current amplitude, voltage dependence and kinetics of activation and inactivation of the channel (González Sánchez et al. 2019). Beta subunits may also play a role in the membrane trafficking of the α1-subunit. At least four genes encoding beta subunits have been uncovered and all are expressed in the brain (Escayg et al. 2000).

The α-subunits can be grouped into three families (1) the high-voltage activated dihydropyridine-sensitive (Cav1.x) channels; (2) the high-voltage activated dihydropyridine-insensitive (Cav2.x) channels and (3) the low-voltage-activated (Cav3.x) channels (Alexander et al. 2011). They can be further classified as L-type (Cav1.1–Cav1.4), P/Q-type (Cav2.1), N-type (Cav2.2), R-type (Cav2.3), and T-type channels (Cav3.1–Cav3.3) (Liu and Wang 2019). Low-voltage channels, like the T-type, differ from high-voltage channels in several ways. T-type channels can be activated and inactivated at low voltages near the resting membrane potential, recover faster from inactivation, deactivate more slowly, and have a characteristic current window occurring in the range of the resting membrane potential of neurons (Perez-Reyes 2003). In fact, during the hyperpolarization phase of the action potential the inactivation of T-type calcium channels is abolished. When the resting membrane potential is recovered, the activation of these channels depolarizes the membrane potential further to trigger rebound firing (Hashiguchi et al. 2019). Thus, they act as pacemakers and excitability regulators (Coutelier et al. 2015). In neurons, they serve two essential functions including triggering of burst action potentials after a low threshold calcium spike and rebound burst firing (Coutelier et al. 2015).

Table 2 Ataxia related to mutations in calcium channel genes

Ion channel name/gene	Ion channel type	Ataxia types	Mode of inheritance/effect of mutation	Age of onset	Additional distinguishing features	Cerebellar atrophy present or not
Cav2.1/ CACNA1A	Voltage-gated	Episodic ataxia type 2	Autosomal dominant/loss-of-function and/or gain-of-function	Infancy or early childhood	Duration of attack: minutes to hours Interictal symptoms can include nystagmus and slowly progressive ataxia Migraines and epilepsy can co-occur	May be present
Cav2.1/ CACNA1A	Voltage-gated	Spinocerebellar ataxia type 6	Autosomal dominant/loss-of-function and/or gain-of-function	Throughout adulthood	Slowly progressive Dysphagia, dystonia, hyperreflexia may be associated	May be present
Cav3.1/ CACNA1G	Voltage-gated	Spinocerebellar ataxia type 42	Autosomal dominant/gain-of-function	Young adulthood Rare early onset form	Young adulthood: slowly progressive Early onset: neurodevelopmental deficits, strabismus, dysmorphism, epilepsy	Present
Cav2.1/ CACNB4	Voltage-gated	Episodic ataxia type 5	Autosomal dominant	Adulthood	Duration of attack: minutes to hours Interictal ataxia, ataxic gait, vertigo, and nystagmus Response to acetazolamide	
IP3R1/ITPR1	Ligand-gated	Spinocerebellar ataxia type 15/16	Autosomal dominant/loss-of-function	Adulthood	Slowly progressive Pyramidal symptoms	May be present
IP3R1/ITPR1	Ligand-gated	Spinocerebellar ataxia type 29	Autosomal dominant/???	Congenital	Nonprogressive and may improve Gross motor delay, mild cognitive impairment	May be present
IP3R1/ITPR1	Ligand-gated	Gillespie syndrome	Autosomal dominant or autosomal recessive/loss-of-function	Congenital	Nonprogressive ataxia Bilateral partial aniridia, gross motor delay, variable intellectual disability	Present
TRPC3	Ligand-gated	Spinocerebellar ataxia type 41	Autosomal dominant/gain-of-function	Adulthood (1 case)		Present

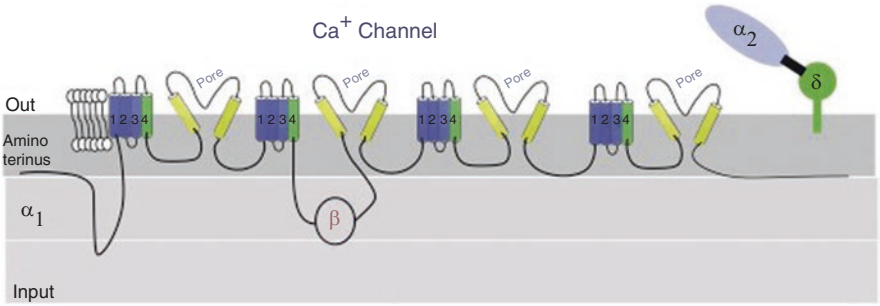

Fig. 3 Voltage-gated calcium channel structure (Mohammad 2020). The channels form hetero-oligomeric complexes containing an α1 subunit with pore-forming domains and auxiliary subunits including β, g and α2–δ (Escayg et al. 2000). The α1 subunit also provides the extracellular binding site for agonists and antagonists (Alexander et al. 2011). Each α1 subunit has four homologous repeats (I–IV), each repeat having transmembrane domains S1–S6 and a pore-forming region between transmembrane domains S5 and S6 (Alexander et al. 2011). Gating is thought to be associated with the membrane spanning S4 segment, which contains highly conserved positive arginine residues that are the voltage sensing elements. The interaction of α1 with these other subunits, including β and α2–δ, influences the properties of the channel (Catterall 2011)

As implied by their name, high-voltage channels activate at higher voltages and inactivate more slowly making the calcium current longer lasting. While L- and T-type channels can be found in a wide range of cell types, N-, P-, Q- and R- type channels are prominently found in neurons (Catterall 2011). For instance, P/Q-type calcium channels are expressed at high levels in granule and Purkinje cells of the cerebellar cortex (Casey and Gomez 2019) (Fig. 3).

4.2 CACNA1A-*Related Ataxia*

Abnormalities of the *CACNA1A* gene on chromosome 19p13 resulting in diverse mutations in the Cav2.1 (α1A) subunit of the voltage-dependent P/Q-type calcium channel have been associated with a wide variety of neurological conditions including familial hemiplegic migraine, epilepsy, cognitive impairment, and congenital or progressive ataxia. Interestingly, there can be clinical overlap between the different conditions which have been called the *CACNA1A*-spectrum disorders.

4.2.1 Episodic Ataxia Type 2 (EA2)

EA2 is an autosomal dominant disorder characterized by paroxysmal episodes of ataxia, characterized by debilitating spells of unsteadiness, incoordination, vertigo, and slurring of speech lasting minutes to hours (Jen and Wan 2018; Giunti et al. 2020). In some cases, patients can have other ictal symptoms including diplopia,

primary position nystagmus, oscillopsia, dystonia, and weakness (Giunti et al. 2020). The majority of patients have onset in infancy or early childhood. Attacks can be triggered by physical exertion, fatigue, emotional distress, or excitement, but can also occur spontaneously (Jen and Wan 2018; Giunti et al. 2020). Interictal symptoms can occur, including persistent nystagmus and slowly progressive ataxia with associated cerebellar atrophy (Giunti et al. 2020). Migraines and epilepsy have also been reported concurrently (Giunti et al. 2020; Verriello et al. 2021). Similarly, patients with the allelic disorder familial hemiplegic migraine Type 1 (FHM1) also may experience episodes of ataxia and even cerebellar atrophy.

Both EA2 and FHM1 have been attributed to heterozygous mutations of the *CACNA1A* gene, known to encode the Cav2.1 (α1A) voltage-gated calcium channel protein. The mutational spectrum of these two disorders and the other members of the *CACNA1A*-spectrum family although distinct, have prominent overlap, which is an area of active study. For EA2 the most commonCav2.1 mutations in *CACNA1A*aredeletions, splice site mutations and premature termination codons (nonsense mutations), while many familial hemiplegic migraine type 1 (FHM1) patients bear missense mutations. Nevertheless, there is prominent genetic and phenotypic overlap among these disorders (Jen and Wan 2018). Exome sequencing has revealed many de novo missense and nonsense mutations in the *CACNA1A* gene in individuals with congenital ataxia or epileptic encephalopathy. EA2 has an extremely variable phenotype even in members of the same family, including twins, who share an identical genotype, raising the possibility of environmental or developmental influences (Giunti et al. 2020). The molecular-cellular pathogenesis of EA2 is still unclear. While some of the mutations expressed in a heterozygous manner in cultured cells exhibit loss-of-function or gain-of-function effects on the Cav2.1 channel, there is not a strong correlation with these distinct channel perturbations and a specific *CACNA1A* clinical syndrome. These findings obviously do not provide insight into therapy. Nevertheless, two drugs, acetazolamide and Fampridine (prolonged 4-aminopyridine or 4-AP), have both been shown to reduce the number of attacks in EA2 when compared to placebo (Muth et al. 2021). Acetazolamide is thought to alter the intracellular pH resulting in a change in the transmembrane potential. 4-AP is thought to act by prolonging the duration of action potentials through blockade of potassium channels, such as Kv1.5, leading to increased excitability of Purkinje cells and increasing release of GABA (Muth et al. 2021).

4.2.2 Spinocerebellar Ataxia Type 6 (SCA6)

SCA6 is characterized by slowly progressive ataxia characterized by ataxic gait, upper extremity incoordination, intention tremor, dysarthria, and nystagmus (vertical and horizontal gaze-evoked). Other features may include diplopia, difficulty fixating on moving objects, dysphagia, dystonia and blepharospasm and hyperreflexia (Casey and Gomez 2019). The age of onset ranges from 19 to 73 years (mean 43 to 52 years) (Casey and Gomez 2019). Imaging studies may reveal cerebellar atrophy, especially vermian atrophy, and pathological studies may reveal both Purkinje and granule cell loss (Casey and Gomez 2019; Gomez et al. 1997).

SCA6 is an autosomal dominantly inherited condition resulting from an abnormal CAG trinucleotide repeat expansion (20–33 repeats) in the *CACNA1A* gene, with some evidence that patients with larger repeats have an earlier age of onset. The CAG repeat encodes a polyglutamine (polyQ) repeat tractin the extreme C terminus of the Cav2.1 (α1A) channel protein. Penetrance is nearly 100% and because of the stability of the small repeat, there is no evidence of anticipation. However, the *CACNA1A* gene is bicistronic, encoding not only the α1A channel protein that serves as the pore-forming subunit of the P/Q calcium channel, but also a transcription factor, α1ACT, which is generated by means of a cellular internal ribosomal entry site (IRES) starting from exon 40 the C terminus-coding end of the α1A mRNA (Du et al. 2013, 2019; Casey and Gomez 2019). The α1ACT protein translocates to the nucleus and acts to enhance expression of genes important for neuronal growth and viability (Du et al. 2013). Both α1A and α1ACT bear the expanded polyQ tracts in SCA6 (Casey and Gomez 2019). There is conflicting evidence regarding whether the expanded polyQ has any effect on the function of the Cav2.1 (α1A) channel, with the most compelling evidence demonstrating that mice expressing Cav2.1 (α1A) channels with expanded tracts have normal P/Q channel function (Du et al. 2019). Thus, attempts to attribute SCA6 to a disturbance in calcium channel function have been unsuccessful. However, wild-type α1ACT with normal polyQ tracts binds and enhances expression of several Purkinje cell-expressed genes, promotes neurite outgrowth, and partially rescues the *CACNA1A* knockout. On the other hand, α1ACT with the expanded polyQ tract reduces viability of cells in vitro and causes gait impairment and cerebellar cortical atrophy in vivo (Du et al. 2013). These characteristics suggest that SCA6 is mediated by α1ACT protein and that the expanded polyQ tract leads to loss-of-function combined with toxic gain-of-function. This novel feature may offer a potential therapeutic target for SCA6 patients that include selectively targeting the *CACNA1A* IRES and preventing expression with specific small molecule inhibitors, microRNAs or antisense oligonucleotides (ASOs) (Miyazaki et al. 2016; Pastor et al. 2018).

4.3 CACNA1G-Related Ataxia/Spinocerebellar Ataxia Type 42 (SCA42)

Like SCA6, SCA42 is also thought to be more of a pure cerebellar ataxia as described initially in the original French and Japanese families (Morino et al. 2015; Coutelier et al. 2015). The age of onset in most families described tends be during young adulthood (Hashiguchi et al. 2019). Ataxia, which is characterized by dysarthria, gait disturbance and abnormal eye movements (saccadic pursuits), is slowly progressive (Coutelier et al. 2015). Other less common reported features include pyramidal signs, truncal myoclonus, tremor, learning disability, dementia and psychiatric manifestations including aggressive behavior and delusions. Brain MRI has revealed moderate cerebellar atrophy and postmortem studies reveal significant loss

of Purkinje cells (Coutelier et al. 2015). More recently, a rare early onset form of SCA42 characterized by neurodevelopmental deficits (developmental delay/intellectual disability; motor, language and social involvement), axial hypotonia/distal hypertonia, ataxia (dysmetria, nystagmus), and strabismus was described (Chemin et al. 2018). In addition, individuals also had dysmorphic features (microcephaly, high frontal hairline, thin hair, broad forehead, deep set eyes, upslanted palpebral fissures, etc.) and digital anomalies (clinodactyly, broad thumbs, broad halluces) (Casas-Alba et al. 2021). Epilepsy is more common to one variant (p.Met153Val) and early-onset epileptic encephalopathy can be an early presentation (Casas-Alba et al. 2021). This subtype manifests within the first year of life (Chemin et al. 2018). MRI demonstrates cerebellar atrophy (global or vermian) and/or hypoplasia (Chemin et al. 2018).

The gene affected in SCA42 is the *CACNA1G* gene on chromosome 17q21. *CACNA1G* encodes for the T-type, low-voltage-activated voltage-gated calcium channel a1G or Cav3.1 (Coutelier et al. 2015). Cav3.1 is highly expressed in cerebellar neurons, including Purkinje Cells, and thalamic relay neurons where these activities are prevalent (Talley et al. 1999). In the more common adult onset form, a heterozygous missense mutation, p.Arg1715His, affecting the S4 voltage-sensing segment of Cav3.1 is inherited in an autosomal dominant fashion (Coutelier et al. 2015; Morino et al. 2015). Activation of mutant Cav3.1 is shifted toward more depolarized or positive membrane potentials, which is important if the complex contains both wild type and mutant proteins, since all four voltage sensors must activate simultaneously in order to open the channel (Morino et al. 2015). The mutation does not seem to affect distribution of or membrane translocation, but the activation and inactivation curves for the calcium currents were shifted positively (Morino et al. 2015). Overall, the change seen is a reduction in the number of spikes per burst and a delayed onset of burst firing leading to an increase in interval between bursts seeming to indicate a decrease in neuronal excitability (Coutelier et al. 2015). In early onset disease, two pathogenic variants have been found de novo, p.Ala961Thr and p.Met1531Val (Casas-Alba et al. 2021). Of the 11 patients with early onset, 8 had the p.Ala961Thr variant (Casas-Alba et al. 2021). Both variants are located within the transmembrane segment S6 contributing to the pore lining of the channel (Chemin et al. 2018). The mutations led to slower inactivation and deactivation kinetics than the wild-type channel, which would result in larger influx of calcium ions into the cell at potentials close to the resting potential. The mutant channels would also remain open longer upon depolarization, both of which correspond to a gain-of-function (Chemin et al. 2018). However, there was also a negative shift in potential in steady state activation and inactivation, which would be consistent with a loss of channel activity as a smaller fraction of Cav3.1 channels would activate in the physiological range of the membrane potential (Chemin et al. 2018). The fact that modeling experiments demonstrated increased spike frequency indicates enhanced firing activity in the deep cerebellar nucleus neurons, which would suggest that overall, the two mutations lead to a gain-of-function despite these differences (Chemin et al. 2018).

4.4 *CACNB4-Related Ataxia/Episodic Ataxia Type 5 (EA5)*

EA5 is a rare cause of episodic ataxia. There are some resemblances with EA2, including similar duration of attacks (can be hours or longer), interictal symptoms (ataxia, ataxic gait, vertigo, and nystagmus), response to acetazolamide with reduction in attacks, and incomplete penetrance (Escayg et al. 2000; González Sánchez et al. 2019). EA5 seems to have later onset, starting in adulthood (Escayg et al. 2000; González Sánchez et al. 2019). Ictal symptoms may include vertigo, dysarthria, dysmetria, and wide-based ataxic gait (Escayg et al. 2000; González Sánchez et al. 2019). Other ictal symptoms described include obtundation, hypoalgesia, and hemiparesis in one patient (González Sánchez et al. 2019). Triggers include lack of sleep, fatigue, alcohol, infections, and psychological stress (González Sánchez et al. 2019).

EA5 has been linked to an autosomal dominantly inherited missense mutation (c.311G>T; p.Cys104Phe) in the voltage-gated calcium channel gene *CACNB4* located on chromosome 2q22–23 (González Sánchez et al. 2019). The B4 subunit is the most highly expressed β subunit in the cerebellum (Escayg et al. 2000). The EA5 mutant did not appear to alter channel kinetics, but substitution of residues may disrupt the conformation and interaction with other proteins including the α1 subunit (Escayg et al. 2000). This in turn may affect trafficking or function in a yet to be determined way resulting in the phenotype seen in EA5 (Escayg et al. 2000).

4.5 *ITPR1-Related Ataxias*

Inositol 1,4,5-trisphosphate (IP3) is an intracellular second messenger generated by cleavage of the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP2) into IP3 and diacylglycerol by phospholipase C. IP3 receptors (IP3Rs) are a group of calcium channels located in the membrane of the endoplasmic reticulum (ER) that play a significant role in intracytoplasmic calcium concentration. Their primary function is to release calcium from the ER into the cytoplasm after binding IP3, thus influencing intracytoplasmic calcium concentration and modulating cellular activities such as axonal transport, level of excitation, synaptic transmission, division, development and apoptosis (Terry et al. 2020; Tada et al. 2016; Keehan et al. 2021). The IP3R1 subtype of IP3 receptors gene is encoded by the gene *ITPR1*. IP3R1 receptors have three functional domains, an IP3 binding domain in the N terminus, a coupling/regulatory domain centrally, and a C-terminal transmembrane spanning pore (Foskett et al. 2007) (Fig. 4). As described below it appears that genetic disruption of these distinct domains in the IP3R1 protein may result in distinct clinical presentations. The prevailing belief is that IP3 binding to the N terminus of the receptor triggers conformational changes that result in opening the channel pore located at the C terminus, but the precise mechanism of this gating is uncertain (Terry et al. 2020). Mutations have been identified in each of these domains and may interfere with various aspects of receptor function including ligand

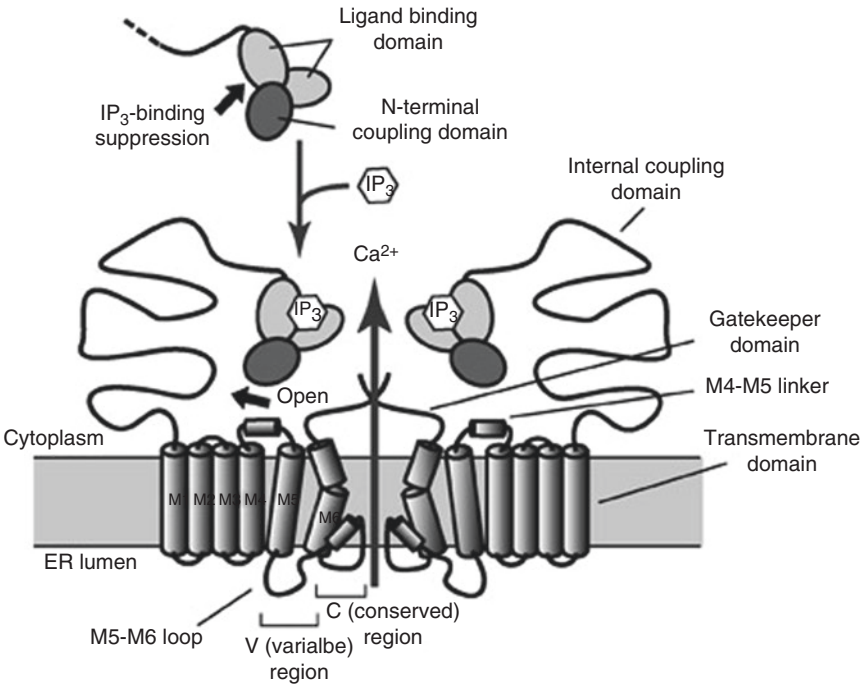

Fig. 4 IP3R channel structure (Yamazaki and Mikoshiba 2009). There are three functional domains: an IP3 binding domain in the N terminus, a coupling/regulatory domain centrally, and a C-terminal transmembrane spanning pore (Foskett et al. 2007). IP3 binding to the N terminus of the receptor triggers conformational changes that result in opening the channel pore located at the C terminus, but the precise mechanism of this gating is uncertain (Terry et al. 2020)

binding, allosteric regulation, ion permeation, protein folding, stability and localization (Terry et al. 2020). However, many of the downstream effects of these mutations are not yet well understood or poorly characterized and the pathogenic mechanism remains to be elucidated in conditions associated with the *ITPR1* gene. The IP3R1 receptor is abundant in cerebellar Purkinje cells, cortex, hippocampus, thalamus, caudate, and putamen (Yamada et al. 1994; Tada et al. 2016). One thought is that reduced levels or excessive activation of IP3R1 may cause disruption of intracellular calcium homeostasis, which in turn causes dysfunction of Purkinje cells and eventually degeneration as well (Tada et al. 2016). Pathogenic mutations in the *ITPR1* gene can cause several ataxic conditions, including spinocerebellar ataxias 15/16 and 29 and Gillespie syndrome (Fig. 4).

4.5.1 Spinocerebellar Ataxia Type 15 (SCA15)

SCA15 typically has onset during adulthood and is slowly progressive. Some individuals retain ambulatory ability multiple decades after onset (Storey 2014). The disease phenotype is variable and common symptoms may include gait ataxia, dysarthria,

limb ataxia, intention and/or postural tremor, head tremor, nystagmus, truncal ataxia, and pyramidal signs (Tipton et al. 2017). Mild dysphagia, mild cognitive impairment, impaired vestibulo-ocular reflex gain, and movement induced oscillopsia have also been reported (Storey 2014). Age of onset typically is age 30–50 years, but ranges from 7 to 72 years, and initial symptoms may include gait ataxia and tremor (Storey 2014; Tada et al. 2016). Imaging may reveal atrophy of the rostral and dorsal vermis of the cerebellum with mild cerebellar hemispheric atrophy (Storey 2014).

SCA15 is an autosomal dominantly inherited condition typically caused by deletions in the *ITPR1* gene, but missense mutations have also been reported (Tipton et al. 2017; Tada et al. 2016). The deletions are of various sizes and include larger deletions encompassing portions or all of the*ITPR1*gene in association with partial deletions in the adjacent SUMF1 gene as well (Storey 2014; Tipton et al. 2017). SUMF1 is associated with the autosomal recessive disease multiple sulfatase deficiency characterized by mental retardation, seizures, and leukodystrophy (Tada et al. 2016). While these individuals are at risk of developing SCA15, they are not at risk of developing the other condition unless the non-deleted homologue of SUMF1 also has a pathogenic variant (Storey 2014). Penetrance is unknown. Given that most pathogenic variants are caused by deletion, anticipation is not likely (Storey 2014). It is presumed that haploinsufficiency and loss of function is the pathogenic mechanism due to the fact that most cases are usually caused by deletion in the gene, but the exact mechanism remains to be elucidated (Storey 2014; Tada et al. 2016; Keehan et al. 2021). In the case of the missense mutation, one hypothesis is that such a change might reduce the level of IP3R1 protein, because studies have shown that the functional properties of the mutated protein are largely unaffected due to the mutation (Tada et al. 2016).

4.5.2 Spinocerebellar Ataxia Type 16 (SCA16)

First described in 2001 in a Japanese family with individuals found to have nystagmus and truncal ataxia, the SCA16 locus was eventually reassigned and a point mutation was found to have overlap with one that was earlier found in SCA15 (Miyoshi et al. 2001; Tipton et al. 2017). Later, another study found that a heterozygous deletion limited to exons 1–48 of *ITPR1* was responsible and indicated again that haploinsufficiency and loss of function were the likely pathological mechanism for what is essentially a genetically identical disorder (Iwaki et al. 2008; Tipton et al. 2017). The suggestion was then to make SCA16 a "vacant SCA" and place this family under the umbrella of SCA15 (Gardner 2008; Tipton et al. 2017).

4.5.3 Spinocerebellar Ataxia Type 29 (SCA29)

SCA29 is a congenital, non-progressive ataxia associated with infantile-onset, hypotonia, gross motor delay (head control, sitting upright, standing, fine motor, speech; delayed by months or years) and mild cognitive impairment (Zambonin

et al. 2017). Impaired ocular fixation and global developmental delay can also be seen (Zambonin et al. 2017). In addition to gait ataxia, the most common cerebellar signs were dysmetria, dysarthria and intention tremor, which were present in over 75% of individuals, but nystagmus, abnormal saccades, oculomotor apraxia, and dysdiadochokinesia were also seen (Zambonin et al. 2017). In one study reviewing the natural course of the disease, improvements across multiple domains including ataxia, tone, and eventual attainment of developmental milestones, speech, coordination, and motor function have been reported, though it is not clear whether or not this was due to early intervention (Zambonin et al. 2017). Cognitive impairment can range from none to moderate impairment (Zambonin et al. 2017). Imaging can show cerebellar atrophy, including superior cerebellar hemispheres and vermis (Zambonin et al. 2017).

SCA29 is autosomal dominantly inherited condition and is usually caused by a heterozygous missense mutation (Zambonin et al. 2017). Some of these missense mutations were thought to affect the coupling/regulatory domain of the *ITPR1* gene product. This domain contains binding sites that act as competitive inhibitors for IP3 and include IRBIT (inositol triphosphate receptor binding protein) and CARP (carbonic anhydrase-related protein VIII), which help modulate IP3R1 activity (Zambonin et al. 2017; Tada et al. 2016). Therefore, these mutations were thought to result in dysregulation of IP3R1 rather than haploinsufficiency by reducing the binding affinities of the IRBIT and CARP proteins and relatively increasing the affinity of IP3 to IP3R1 leading to exaggeration of the pathway mediated by IP3R1 (Zambonin et al. 2017; Tada et al. 2016). This may imply a gain of function rather than loss (Casey et al. 2017). However, some mutations also affect the transmembrane region as well (Zambonin et al. 2017). If the pathogenic mechanism was loss of function in this case, then one may hypothesize that the mutation has a dominant-negative effect on the function of the IP3R1 complex (Tada et al. 2016). A more recent study had shown one particular mutation resulted in alteration of the binding domain with replacement of a positively charged arginine residue with a neutral tryptophan residue, thus decreasing affinity for the negatively charged IP3 molecule (Terry et al. 2020).

4.5.4 Gillespie Syndrome (GS)

GS is a rare congenital disorder first described by Gillespie in 1965. One of the most striking and consistent features is bilateral partial aniridia resulting in a fixed and large pupil (Keehan et al. 2021). Iris hypoplasia results in scalloped or "festooned" edges at the pupillary border with iris strands extending onto the anterior lens surface at regular intervals (Hall et al. 2019). Other common characteristics include congenital hypotonia, nonprogressive ataxia (including gait and balance impairment, incoordination, intention tremor, and scanning speech), delay in meeting motor milestones and varying degrees of intellectual disability (Keehan et al. 2021; Gerber et al. 2016; McEntagart et al. 2016). Other reported features in patients identified as having GS include facial dysmorphism, cardiac defects including

pulmonary valve stenosis and patent foramen ovale and gastrointestinal defects, including intestinal malrotation (Hall et al. 2019; Paganini et al. 2018; McEntagart et al. 2016; Carvalho et al. 2018). Imaging studies have shown cerebellar atrophy/hypoplasia, including vermian and superior cerebellar atrophy (Stendel et al. 2019; McEntagart et al. 2016).

The mode of inheritance for GS includes both autosomal recessive and dominant mutations in *ITPR1*. Causative variants include single-nucleotide missense mutations and deletions that cluster frequently near or within the C-terminal transmembrane channel domain of the gene thus affecting ion transport (Keehan et al. 2021; Paganini et al. 2018; Hall et al. 2019). As an example, codon Lys2569 deletion resulted in decreased calcium release activity in mutant transfected cells, but this has not been demonstrated in vivo (Hall et al. 2019; Gerber et al. 2016). Variants have also been identified in the central regulatory domain (Paganini et al. 2018; Stendel et al. 2019). Dominant mutations, which can be inherited or de novo, are thought to result in a dominant-negative effect with the prevailing model suggesting that they compromise the homotetrameric structure of the channel and thus the normal functioning of the pore (Paganini et al. 2018; Dentici et al. 2017; Gerber et al. 2016; McEntagart et al. 2016; Keehan et al. 2021; Hall et al. 2019). Recessive mutations are homozygous or compound heterozygous and result in truncation due to generation of premature stop codons. This leads to complete or occasionally partial loss-of-function with possible persistence of small amounts of truncated protein (Hall et al. 2019; Paganini et al. 2018). The severity of the disease may then be modulated by the position of the mutations, presence and amount of wild-type protein and the ability of mutated proteins to be incorporated without effecting the overall function of the channel (Paganini et al. 2018). To the contrary, one study found that only wild-type homotetramers were able to contribute a significant amount of calcium release, and if tetramers form without bias leading to a normal distribution of mutant and wild-type subunits in the tetramer, the reduced fraction of fully wild-type homotetramers would result in attenuated calcium release (Terry et al. 2020).

4.6 TRPC3-Related Ataxia/Spinocerebellar Ataxia Type 41 (SCA41)

The first and only confirmed case of what is thought to be SCA41 was described recently. The patient was a 40-year-old white male of European ancestry who presented with 2 years of progressive imbalance and ataxic gait (Fogel et al. 2015). He had an extensive and unremarkable evaluation for acquired causes of ataxia, dominantly inherited ataxias, and there was no obvious family history, but he was estranged from his father (Fogel et al. 2015). His MRI brain showed mild vermian atrophy (Fogel et al. 2015). Exome sequencing revealed a single variant of potential clinical significance, which was a heterozygous point mutation (p.Arg762His; 122824185G>A) of the *TRPC3* gene located on chromosome 4 (Fogel et al. 2015).

This position is highly conserved and the protein change was predicted to be damaging. His known family was unaffected and the variant was not found maternally, but they were unable to determine if it was inherited or de novo due to lack of paternal data and therefore could not directly confirm pathogenicity (Fogel et al. 2015).

TRPC3 is a part of the transient receptor potential family, which is expressed in Purkinje cells of the cerebellum even early in development (Becker 2017; Fogel et al. 2015). The gene encodes a non-selective cation channel permeable to sodium and calcium linked to key signaling pathways and synaptic transmission in Purkinje cells including the one mediated by metabotropic glutamate receptor subtype 1 (mGluR1) (Becker 2017). Activation results in calcium influx, but permeability to sodium might also activate voltage-gated calcium channels through changes in membrane potential (Becker 2017). The p.Arg762His variant is located within a highly conserved region implicated in regulating channel gating and a mutation would likely have a significant effect on function (Becker 2017). Toxic gain-of-function is the suspected mechanism of pathological dysfunction in SCA41. Mutant p.Arg762His channels were expressed in similar numbers to the wild-type TRPC3 at the plasma membrane in mouse models, but significantly induced neuronal cell death (Becker 2017). One sign that implicated increased channel activity was significantly increased nuclear localization of the calcium-sensitive transcription factor NFAT in models where there was overexpression of the TRPC3 mutant (Becker 2017). The absence of ataxia symptoms in individuals with heterozygous deletions and rare nonsense variants in the population further supports the theory of toxic gain-of-function (Becker 2017). In mouse models, Purkinje cell loss and impairment in dendritic arborization during cerebellar development result from loss of TRPC3 or from point mutations that cause gain of function (Becker 2017). Purkinje cell firing is markedly abnormal in these models characterized by a depolarization block of spiking and alteration of intrinsic firing frequency (Bushart and Shakkottai 2019).

5 Ataxia Related to Mutations in Sodium Channel Genes

5.1 Ataxia Related to Voltage-Gated Sodium Channels

Sodium channels initiate action potentials in neurons and excitable cells (Catterall 2018; Wagnon et al. 2018; O'Brien and Meisler 2013) (Table 3). The channels consist of a large central pore-forming α subunit in complex with one or two auxiliary β subunits that may be involved in the trafficking of α subunits (Catterall 2018) (Fig. 5). α-Subunits consist of four homologous domains (I–IV), each containing six transmembrane segments (S1–S6) and a pore-forming loop. (Alexander et al. 2011) The positively charged fourth transmembrane segment (S4) acts as a voltage sensor and is involved in channel gating (Alexander et al. 2011). The S5 and S6 segments combine with the connecting pore-loops (P-loops) to form the channel pore

Table 3 Ataxia related to mutations in sodium channel genes

Ion channel name/ gene	Ion channel type	Ataxia type	Mode of inheritance/ effect of mutation	Age of onset	Additional distinguishing features	Cerebellar atrophy present or not
Nav1.1/ SCN1A	Voltage-gated	Dravet syndrome	Autosomal dominant/loss-of-function	Infantile onset	Drug-resistant epilepsy, developmental delay, moderate to severe intellectual disability, parkinsonism, high risk of early mortality from SUDEP	May be present
NaV1.2/ SCN2A	Voltage-gated	Episodic ataxia	Autosomal dominant/gain-of-function	Early onset (10 months to 14 years)	Seizures early in life During ataxic episodes, encephalopathy, dystonic posturing, myoclonus, vomiting, hyperreflexia can be present Weekly to monthly episodes in most cases with episodes typically lasting minutes to hours	May be present
Nav1.6/ SCN8A	Voltage-gated		Autosomal dominant/gain-of-function or loss-of-function		May range from developmental and epileptic encephalopathy (DEE; most common presentation) to benign epilepsy Intellectual disability, autism, ataxia, myoclonus, choreoathetosis, paroxysmal dyskinesia may coexist or occur independent of seizures	May be present

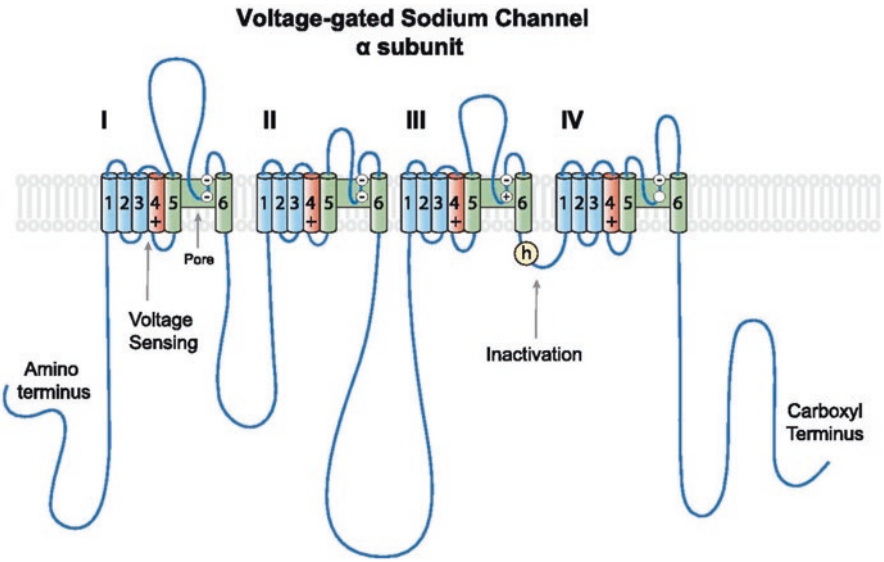

Fig. 5 Voltage-gated sodium channel structure (Denomme et al. 2019). Channels consist of a large central pore-forming α subunit in complex with one or two auxiliary β subunits that may be involved in the trafficking of α subunits (Catterall 2018). α-Subunits consist of homologous domains I–IV, each containing transmembrane segments S1–S6 and a pore-forming loop (Alexander et al. 2011) The positively charged S4 segment acts as a voltage sensor and is involved in channel gating (Alexander et al. 2011). The S5 and S6 segments combine with the connecting pore-loops (P-loops) to form the channel pore and ion-selectivity filter (Reynolds et al. 2020). Connecting loops link the transmembrane segments, larger intracellular loops link the four homologous domains, and the intracellular loop between domains III and IV is the fast-inactivation gate (Reynolds et al. 2020). Membrane depolarization facilitates a conformational change in the channel which leads to channel activation, movement of the positively charged S4 voltage-segment domain and opening of the ion selectivity pore, allowing sodium influx (Reynolds et al. 2020). During depolarization, the channel inactivates during the fast inactivation phase by the inactivation gate folding into and occluding the channel pore (Reynolds et al. 2020)

and ion-selectivity filter (Reynolds et al. 2020). Connecting loops link the transmembrane segments, larger intracellular loops link the four homologous domains, and the intracellular loop between domains III and IV is the fast-inactivation gate (Reynolds et al. 2020). Membrane depolarization facilitates a conformational change in the channel which leads to channel activation, movement of the positively charged S4 voltage-segment domain and opening of the ion selectivity pore, allowing sodium influx (Reynolds et al. 2020). During depolarization, the channel inactivates during the fast inactivation phase by the inactivation gate folding into and occluding the channel pore (Reynolds et al. 2020). Hyperpolarization releases the inactivation gate and enables channel activation once again (Reynolds et al. 2020). Sodium channels rapidly activate, open and inactivate in response to depolarizing stimuli and an additional slow inactivation process is engaged by long trains of stimuli or prolonged depolarization (Catterall 2018).

5.2 SCN1A-Related Ataxia/Dravet Syndrome

Dravet Syndrome (DS) is a rare infantile onset, chronic epileptic encephalopathy characterized by drug-resistant epilepsy, developmental delay, and high risk of early mortality (17% by 20 years of age) mainly due to sudden unexpected death in epilepsy (SUDEP) or status epilepticus (SE). DS may represent 2–3% of children with refractory epilepsy who transition to adult care (Andrade et al. 2021). Fever or hyperthermia may trigger the initial seizure, which typically occurs before age 19 months (Andrade et al. 2021). Seizure types are variable and can include GTCs, myoclonic, absence, focal impaired seizures and others. Seizures can be prolonged, cluster or result in SE (Andrade et al. 2021). Moderate to severe intellectual disability is common and individuals can regress and lose acquired skills following a prolonged seizure or episode of SE (Andrade et al. 2021). Autism can also co-occur with DS (Andrade et al. 2021). Behavioral abnormalities include attention deficit disorder, agitation, irritability, and aggressiveness (Andrade et al. 2021). Patients who are ambulatory may develop a wide-based ataxic gait with other features developing as they age including crouching, camptocormia, dystonic posturing, tiptoeing, and parkinsonian quality (Andrade et al. 2021). Cerebellar speech patterns and other features of parkinsonism, including cogwheeling rigidity and bradykinesia, along with anterocollis may also be present. Parkinsonism can be levodopa responsive (Andrade et al. 2021).

80–90% of patients with a clinical diagnosis of DS have a pathogenic variant in the *SCN1A* gene which encodes the α1 subunit of the neuronal voltage-gated sodium channel Nav1.1 expressed in GABAergic interneurons (Andrade et al. 2021; Catterall 2018; Gataullina and Dulac 2017). DS is an autosomal dominant disorder primarily caused by heterozygous, de novo loss-of-function mutations involving Nav1.1, but germline mosaicism or somatic mosaicism have also been reported (Catterall 2018; Andrade et al. 2021). Half the variants have mutations leading to truncation and reduced protein expression and the others have missense mutations in the pore forming part or the voltage sensor part leading to haploinsufficiency (Scheffer and Nabbout 2019; Gataullina and Dulac 2017). The loss of function of these sodium channels may explain why sodium channel blocking anti-epileptic drugs exacerbate seizures in DS. Variants may alter Nav1.1 activation as well as the slow inactivation of interneurons (Layer et al. 2021). Mutant channels may open and reach their maximal activation at more depolarized potentials (Layer et al. 2021). Entry into slow inactivation was accelerated and voltage dependence was shifted to more hyperpolarized potentials in one study (Layer et al. 2021). The loss-of-function mutations had the specific effect of reducing the sodium currents and electrical excitability of GABAergic interneurons, which decreases inhibitory input and leads to overall hyperexcitability throughout the brain at baseline predisposing to seizures (Catterall 2018). On the other hand, ataxia is thought to occur due to failure of action potential firing in GABAergic Purkinje neurons (Catterall 2018).

5.3 SCN2A-Related Ataxia

SCN2A mutations have been associated with a wide spectrum of clinical presentations including benign familial neonatal seizures (BFNIS), developmental and epileptic encephalopathy (DEE), autism-spectrum disorders, intellectual disability and rarely, episodic ataxia (EA) (Passi and Mohammad 2021). One group reviewed the 21 cases associated with EA in the literature that had been reported up to that point (Schwarz et al. 2019). EA onset ranged from 10 months to 14 years, but was usually early onset (Schwarz et al. 2019). The frequency of EA episodes is weekly to monthly in most cases with episodes typically lasting minutes to hours, but ranged from brief, daily events to 1–2 episodes per year each lasting several weeks (Schwarz et al. 2019). Reported ataxic features included dysarthria, poor balance, hypotonia, and additional features during events included encephalopathy, dystonic posturing, myoclonus, vomiting, hyperreflexia, and tremor (Schwarz et al. 2019; Passi and Mohammad 2021; Liao et al. 2010a). Triggers included stress, sleep deprivation, head trauma, and various sensory stimuli. The large majority of these patients also had concurrent seizures that had onset usually within the first 3 months of life, often during the neonatal stage and on the milder side (Schwarz et al. 2019). Cognitive outcomes were mostly favorable with most patients having normal or mild impairment in most cases with rare exceptions (Schwarz et al. 2019). Cerebellar atrophy was reported in a few individuals, but it was not a consistent feature.

SCN2A-associated EA is often due to de novo mutations, but familial cases have also been found (Schwarz et al. 2019). They are most often the result of heterozygous missense mutations and autosomal dominant (Passi and Mohammad 2021; Schwarz et al. 2019). *SCN2A* encodes the alpha subunit of the voltage-gated sodium channel Nav1.2, which is highly expressed in unmyelinated parallel fibers, which are the axons of granule cells in the molecular layer that project to Purkinje neurons, and the axon initial segments and nodes of Ranvier of myelinated nerve fibers of hippocampal and cortical excitatory neurons (Liao et al. 2010a; Wolff et al. 2019). The prevalence of these channels may vary during different periods of development in the different neuronal types, which may explain why seizures have earlier onset than EA in affected patients and even remit in some cases (Liao et al. 2010a; Schwarz et al. 2019). Nav1.2 is still highly expressed in the dendrites of adult hippocampal and cortical neurons, but, as patients age, it may be replaced by Nav1.6 in the region of the axon initial segment (Liao et al. 2010b). While a number of mutations have been found in association with *SCN2A*-associated EA, there appear to be two mutational hotspots. One common mutational hotspot resulted in p.Ala263Val missense mutation affecting the S5 segment of the domain I in the Nav1.2 alpha subunit (Schwarz et al. 2019). Another hotspot for pathogenic variants is the S4 segment and its cytoplasmic loop within domain IV (Schwarz et al. 2019). This would affect gating of the channel as well as inactivation. The various mutations associated with EA are all thought to lead to a gain-of-function, which may lead to increased sodium current in a variety of ways. These include a hyperpolarizing shift, meaning channels open at voltages closer to resting membrane potential and faster activation,

a depolarizing shift with more channels available for activation at resting membrane potential, slower inactivation, or accelerated recovery after fast inactivation with shortened refractory period after an action potential (Hedrich et al. 2019). Such changes would then lead to an increased persistent sodium current with downstream effects of membrane depolarization in neurons, amplification of synaptic potentials, generation of subthreshold oscillations, and facilitation of repetitive firing maintaining prolonged depolarized plateau potentials (Hedrich et al. 2019). This state of hyperexcitability would understandably lead to susceptibility to seizures. Given this knowledge, sodium channel blockers, such as phenytoin, are the anti-convulsants of choice and are indeed beneficial for the treatment of seizures (Schwarz et al. 2019). However, these drugs were less effective and often not helpful for EA for unclear reasons (Schwarz et al. 2019). Acetazolamide, which has been of benefit for forms of EA, had benefit in a few of the patients in improving frequency and severity (Schwarz et al. 2019).

Interestingly, loss-of-function variants in the SCN2A gene, which are associated with autism spectrum disorder and intellectual disability, also result in epilepsy in 20–30% of affected children (Spratt et al. 2021; Zhang et al. 2021b). While sodium channel loss in excitatory cells would be expected to have the opposite effect, pyramidal neurons in mice lacking Nav1.2 channels were found to be hyperexcitable (Spratt et al. 2021; Zhang et al. 2021b). Downregulation or dysfunction of potassium channels in affected individuals prevented proper repolarization between action potentials allowing neurons to reach the threshold for action potential generation more rapidly, increasing susceptibility to seizures (Spratt et al. 2021; Zhang et al. 2021b).

5.4 SCN8A-Related Ataxia

Ataxia has also been associated with mutations of the *SCN8A* gene, but it may not be the sole, initial or primary manifestation. The phenotype and severity of presentation of *SCN8A*-related disorders is variable and is rapidly increasing in recognition with more widespread testing and whole exome sequencing. Mutations in *SCN8A* have been implicated conditions ranging from developmental and epileptic encephalopathy (DEE) to benign epilepsy. Neurological features such as intellectual disability, autism, and movement disorders, including ataxia, myoclonus, choreoathetosis, and paroxysmal dyskinesia, may coexist or occur independent of seizures in *SCN8A*-related disorders (Gardella and Møller 2019; Larsen et al. 2015). DEE is the most common phenotypic presentation and is characterized by early onset seizures that are often drug-resistant (43 days to 4 months), severe to profound intellectual disability, absent speech, progressive pyramidal and extrapyramidal signs (myoclonus, dystonia, dyskinesia), axial hypotonia and tetraparesis with progressive cerebral atrophy, and cortical blindness (Gardella and Møller 2019). Less frequently *SCN8A* mutations are implicated in benign familial infantile seizures associated with paroxysmal kinesigenic dyskinesia (PKD) (Gardella and Møller

2019). In this presentation, seizures are typically self-limiting and occur during the first year of life and 33% developed PKD in teens (Gardella and Møller 2019). In between these two phenotypes exists an intermediate form characterized by a milder epilepsy phenotype than DEE, normal cognition to moderate intellectual disability, and mild or absent neurological deficits (Gardella and Møller 2019). The mean age of epilepsy onset was 14 months and a majority of patients achieved seizure freedom by age 4–10 years on pharmacological agents (Gardella and Møller 2019). ADHD and autistic traits were observed in some and varying degrees of gait disturbances, ataxia, tremor/myoclonus, hypotonia, movement disorders, and sleep disorders were also reported (Gardella and Møller 2019). A small number of patients have *SCN8A* variants without epilepsy and phenotypic presentations have included mild to moderate intellectual disability with comorbid behavioral symptoms (autism, ADHD, etc.) and discrete neurological symptoms including ataxia, gait instability, hypotonia, speech delay, dyskinesia, tremor, chorea, and myoclonus (Gardella and Møller 2019). The true prevalence of this subtype may be unknown since *SCN8A* mutations may have gone undetected for these sporadic cases (Gardella and Møller 2019). A 2006 case report commented on a 9-year-old boy with intellectual delay, ADHD, motor delay, ataxia, and pancerebellar atrophy with individuals in his family sharing the same mutation and various other manifestations including cognitive impairment and ADHD (Trudeau et al. 2006).

SCN8A-related conditions are autosomal dominant disorders. The gene, located on chromosome 12q13, encodes the α subunit of Nav1.6, which is a voltage-gated sodium channel located in the initial segment of the axon involved in the initiation and propagation of action potentials as well as in the nodes of Ranvierin-myelinated neurons (Gardella and Møller 2019; Wagnon et al. 2018). Nav1.6 is also thought to be important in the generation of the persistent and resurgent currents necessary for rapidly firing neurons such as Purkinje cells (O'Brien and Meisler 2013). Nav1.6 is widely expressed in the brain and is highly expressed in Purkinje cells (Gardella and Møller 2019; Wagnon et al. 2018). SCN pathogenic variants are typically missense mutations in the highly conserved transmembrane domain and tend to be de novo heterozygous mutations, but rarely are inherited due to mosaicism in an unaffected parent (Gardella and Møller 2019). Mutations in the inactivation gait and the cytoplasmic C terminal domain have also been found (Gardella and Møller 2019; Meisler 2019). Gain-of-function mutations have been associated with epilepsy syndromes and are characterized by elevation in sodium currents due to hyperactivity of Nav1.6 as a result of altered voltage dependence leading to premature channel opening or closing, delayed channel inactivation, or increased resurgent or persistent current that leads to increased neuronal firing and excitation (Gardella and Møller 2019; Meisler 2019; Larsen et al. 2015). The properties of these mutations may explain why sodium channel blockers may have more positive outcomes than other types of anti-epileptic drugs with the exception of drug-resistant DEE (Gardella and Møller 2019; Larsen et al. 2015). Loss-of-function mutations are more commonly associated with intellectual disability, autism, and movement disorders, such as ataxia and myoclonus, without seizures (Gardella and Møller 2019). These include protein truncation mutations (C terminal domain in

ataxic patient above), shifted voltage dependence of activation, delayed channel inactivation, impaired trafficking to initial segment of axon, and complete or partial loss of channel activity resulting in reduced neuronal excitability in mouse models (Meisler 2019; Larsen et al. 2015; Trudeau et al. 2006; Wagnon et al. 2018; O'Brien and Meisler 2013). Genotype-phenotype correlation is not straightforward and often results in a spectrum of disorders even with the same mutation (Larsen et al. 2015).

6 Ataxia Related to Mutations Genes Encoding Na+/K+ ATPase

6.1 ATP1A3-Related Ataxia/CAPOS Syndrome

CAPOS syndrome is classically characterized by early onset cerebellar ataxia with a relapsing course, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (Salles et al. 2021). The initial episode follows a febrile illness and usually has onset between 1 and 5 years of age (Salles et al. 2021). Symptoms include acute onset cerebellar ataxia, encephalopathic features, hypotonia, areflexia and weakness (Salles et al. 2021). Other less common symptoms include paresis and transient impairment of hearing and vision (Salles et al. 2021). Most patients recover completely after this initial event, but some have residual ataxic symptoms (Salles et al. 2021). Patients may then have two to three more episodes with a degree of recovery before transitioning to a slowly progressive chronic condition (Salles et al. 2021). In addition to ataxia, patients develop sensorineural hearing loss that may seem acute in onset and progressive, bilateral optic atrophy due to optic neuropathy that leads to loss of vision, poor color discrimination, and diminished brightness sensitivity, nystagmus and strabismus (Salles et al. 2021). Less common symptoms associated with CAPOS include urinary urgency, cardiac arrhythmia, left ventricular enlargement, scoliosis, cognitive dysfunction, autistic traits, bradykinesia, myoclonus, chorea, tremor, oral dyskinesias, and dystonia (Salles et al. 2021). While areflexia is agreed upon as a characteristic of CAPOS, pes cavus is a more controversial symptom as one group remarked that the prevalence was only slightly higher than the general population (Heimer et al. 2015). There are also two related conditions attributed to mutations in *ATP1A3*, rapid-onset dystonia parkinsonism (RDP) and alternating hemiplegia of childhood (AHC). These are now thought to exist on a spectrum of disorders with CAPOS, with both clinical overlap unique manifestations, such as adult-onset relapsing encephalopathy with cerebellar ataxia, adult-onset cerebellar ataxia, fever-induced paroxysmal weakness and encephalopathy, paroxysmal non-kinesigenic dyskinesia (Salles and Fernandez 2020; Salles et al. 2021).

CAPOS syndrome is inherited in an autosomal dominant fashion and is due to a heterozygous missense mutation, p.Glu818Lys, in the *ATP1A3* gene on

chromosome 19q (Demos et al. 2014). The *ATP1A3* gene encodes the α3 isoform, which is one of four α isoforms (α1–4) in the heterotrimeric α-β-γ protein complex that constitutes the Na+/K+ ATPase (Salles and Fernandez 2020; Salles et al. 2021). The Na+/K+ ATPase is a transmembrane ion-pump that extrudes three sodium ions in exchange for two potassium ions into the cell for every adenosine triphosphate utilized. The pump helps to maintain and regulate the electrochemical gradient and is critical for action potential propagation during depolarization (Salles et al. 2021). Thus, even though it is not an ion channel per se, its function is critical to creating the gradient required for other ion channels to function properly. The α3 isoform is mainly expressed in neurons, especially in the basal ganglia, substantia nigra, red nucleus, thalamus, cerebellum, oculomotor nucleus, reticulo-tegmental nucleus of the pons, hippocampus, retina, spiral ganglion, and organ of Corti (Salles and Fernandez 2020). The α3 isoform acts as a rescue pump after repeated action potentials for rapid restoration of large transient increases in intracellular sodium ion concentration (Salles and Fernandez 2020). It may also support reuptake of neurotransmitters (Salles et al. 2021). The p.Glu818Lys mutation is thought to reduce the sodium ion affinity of both the internal and external cation-binding sites (external more so than internal) (Roenn et al. 2019). The reduced sodium ion affinity at the internal sites leads to delayed clearing of the accumulated sodium after an action potential (Roenn et al. 2019). The mutant Na+/K+ ATPase possesses a weaker voltage dependence and stronger potassium ion inhibition, which reduces the inability to rapidly regain the resting membrane potential following action potentials (Roenn et al. 2019). While this model may suggest a loss-of-function mechanism, an alternative theory points to a temperature-sensitive gain-of-function mechanism (Salles et al. 2021).

7 Other Ion Channel Disorders

7.1 SLC1A3-Related Ataxia/Episodic Ataxia Type 6 (EA6)

EA6 due to mutations in the gene, *SLC1A3*, is a rare cause of episodic ataxia with only a few families described up to this point. In addition to paroxysmal and intermittent cerebellar dysfunction, individuals may also experience seizures and migraine-like headaches (Chivukula et al. 2020). Physical and emotional stress, heat, caffeine, alcohol, febrile illness and smoking were some factors seen to trigger events (Choi et al. 2017b). Patients who have EA6 tend to have longer lasting attacks (several hours to days) and lack other features found in other EAs such as myokymia and tinnitus (Chivukula et al. 2020). Patient phenotype and severity may vary depending on mutation. The most severe phenotype is associated with the heterozygous missense mutation p.Pro290Arg (p.P290R) mutation was characterized by early onset severe episodic and progressive ataxia, cerebellar atrophy, seizures, alternating hemiplegia, and migraine (Jen et al. 2005; Chivukula et al. 2020). Interictal symptoms included

hyperreflexia, saccadic pursuits, impaired optokinetic nystagmus, and truncal ataxia (Jen et al. 2005). p.Met128Arg (p.M128R) was associated with onset at 11 months with episodic truncal ataxia, dysarthria, tremor, and strabismus (Iwama et al. 2018). Acetazolamide was effective for her symptoms (Iwama et al. 2018). Another family of 3 patients with the p.Cys186Ser (p.C186S) mutation presented with episodes of ataxia with nausea, photophobia, vertigo, slurred speech, and diplopia/blurred vision from early childhood and interictal gaze evoked nystagmus that was acetazolamide-responsive, but one family member who carried the mutation remained unaffected (De Vries et al. 2009; Chivukula et al. 2020). Another family with the mutation, p.Val393Ile (p.V393I), had documented episodes of unsteadiness and dizziness with late onset (age 55) with eventual development of truncal ataxia and slurred speech with interictal symptoms including nystagmus and abnormal saccades (Choi et al. 2017b). This family had two affected and two unaffected family members with the mutation and affected individuals were acetazolamide responsive (Choi et al. 2017b). The pThr318Ala (p.T318A) mutation was found in patients with ataxia, dizziness and dysarthria. (Chivukula et al. 2020). The last described pathogenic missense variant in this gene, p.Arg454Gln (p.R499Q), was discovered during exome sequencing of undiagnosed inherited ataxias and was characterized by upper limb ataxia, dysarthria, abnormal saccades, and dysphagia that was continuous and progressive (Pyle et al. 2015; Chivukula et al. 2020).

EA6 has autosomal dominant inheritance with both familial and de novo cases recognized. There is also suggestion of incomplete penetrance as there were unaffected carriers in certain families as noted above. The gene affected is the *SLC1A3* which encodes the glial excitatory amino acid transporter 1 (EAAT1) that helps to clear glutamate from the synaptic cleft and regulates its concentration at excitatory synapses in the cerebellum, brainstem and thalamus, while also functioning as an anion channel (Chivukula et al. 2020; Iwama et al. 2018; Winter et al. 2012). EAAT1 contain eight α-helical transmembrane domains (TMD) and re-entrant hairpin loops (HP) 1 and 2 flanking TMD7 (Choi et al. 2017b). The first six TMDs form a scaffold that surrounds a C-terminal core domain comprising HP1, TMD7, HP2 and TMD8 (Choi et al. 2017b). The C-terminal domain is known to play an important role in transporting glutamate by inducing conformational rearrangements (Choi et al. 2017b). Among them, TMD7 is critical binding site for glutamate as well sodium, hydrogen and potassium ions (Choi et al. 2017b). Furthermore, several residues in TMD7 contribute to anion permeation and selectivity (Choi et al. 2017b). EAATs are trimeric proteins with three subunits associating via immobile trimerization domains (Chivukula et al. 2020). Each subunit contains a mobile transport domain with substrate-binding sites that shuttle substrates in both directions and they function independently (Chivukula et al. 2020). This may suggest that a dominant negative effect resulting from the combination of wild type and mutant proteins may be less likely the pathogenic mechanism but could result in impaired trafficking due to retention of these heterotrimers (Chivukula et al. 2020). The p.P290R mutation is located within the trimerization domain, but all other known mutations are located in the transport domain (Chivukula et al. 2020). EAATs act as secondary-active glutamate transporters and anion channels and EAAT1 specifically transports

glutamate stoichiometrically coupled to three sodium ions and one hydrogen ion in exchange for one potassium ion (Chivukula et al. 2020). The anion channel portion opens upon lateral movement of the transport domain from intermediate translocation states, tightly linking anion currents to transitions within the glutamate uptake cycle (Chivukula et al. 2020; Choi et al. 2017b). In other words, glutamate movement may gate the anion channel and anion currents may limit glutamate release (Choi et al. 2017a). As noted above, the mutations lead to variable phenotypes with differences in severity and associated features. The p.P290R mutation, which seems to be the most severe and best characterized, was shown to impair glutamate transport and form gain-of-function of anion channels by increasing probability of opening of the channel (Chivukula et al. 2020). A mouse model demonstrated that excessive chloride ion efflux led to glial apoptosis and cerebellar atrophy early in development (Chivukula et al. 2020). The hypothesis was that such changes would result in an increase in the force driving GABA transporters for reuptake of GABA and then reduce inhibitory synaptic transmission in EA6 patients (Chivukula et al. 2020; Winter et al. 2012). Degeneration of glia would ultimately impair and reduce glutamate reuptake leading to increased glutamate-driven excitation, modify synaptic transmission in the cerebellum and lead to cerebellar degeneration in these animals (Chivukula et al. 2020; Winter et al. 2012). One group studied the potential mechanisms of the other mutations using heterologous expression in mammalian HEK293T cells. The p.M128R variant predicted a 50% reduction in EAAT1 glutamate transport and anion current suggestive of a loss of function of homotrimeric transporters and possible dominant negative effect in heterotrimeric ones. As noted, this model may be controversial given that these subunits are thought to function independently with impaired trafficking the more likely mechanism (Chivukula et al. 2020). On the other hand, the p.T318A mutation caused increased glutamate uptake and anion current amplitude twofold due to increased expression of the mutant protein (Chivukula et al. 2020). The other mutations were thought to have less drastic effects and mechanisms may include impairment of the cotransport process of glutamate due to abnormality in binding sodium ions (p.V393I), mild or even no reduction in glutamate uptake and anion currents, and increased expression of mutants with impairment of late steps in membrane surface insertion of EAAT1leading to it being in close proximity to the membrane instead of being properly inserted, which would reduce macroscopic glutamate uptake and anion currents (Chivukula et al. 2020).

7.2 Ataxia Related to Mutations in ANO10/Autosomal Recessive Cerebellar Ataxia Type 3 (ARCA3)

ARCA3, also known as spinocerebellar ataxia recessive type 10 (SCAR10), is a rare recessively inherited ataxia. The disease is characterized by slowly progressive spastic ataxia (limb ataxia, dysarthria, nystagmus, saccadic abnormalities, ataxic

gait) variably associated with motor neuron involvement and pyramidal signs (hyperreflexia), epilepsy, and cognitive decline (Nanetti et al. 2019). The age of onset is usually in late teens and early adulthood (Nanetti et al. 2019; Yang et al. 2020). Cerebellar atrophy is seen on MRI (Nanetti et al. 2019). Bradykinesia/parkinsonism, mild vertical gaze paresis, pes cavus, and sphincteric disturbances have also been reported (Nanetti et al. 2019). Additional features may include cognitive decline, reduced levels of coenzyme Q10, and elevated serum alpha-fetoprotein (Nanetti et al. 2019). Cognitive impairment is not thought to be common. Executive function impairment can be seen even in those with reportedly normal cognition otherwise (Nanetti et al. 2019).

ARCA3 is the result of mutations involving the gene, *ANO10*, located on chromosome 3p21.33. This gene encodes an eight transmembrane protein named anoctamin 10, which is a *putative* member of a family of calcium-activated chloride channels (Nanetti et al. 2019). *ANO10* expression is mostly found in the adult brain and is especially high in the cerebellum, frontal, and occipital cortices (Vermeer et al. 2010). Individuals may carry either homozygous or compound heterozygous mutations that include missense, nonsense/truncation and deletion (Nanetti et al. 2019). While missense mutations are most common, the c.132dupA mutation is regarded to be the most common found in heterozygosity and leads to a frame shift, introducing a premature stop codon (Nieto et al. 2019). It is known that calcium-activated chlorine channels have important functions including regulation of neuronal excitability (Vermeer et al. 2010). However, the exact function of anoctamin 10 and pathogenesis of mutations remains unclear. It could well be that the *ANO10* gene product, in addition to the function of calcium-dependent chloride channel, could also influence calcium signaling in Purkinje cells, and a dysfunctional or absent anoctamin 10 may cause cerebellar ataxia via this mechanism (Vermeer et al. 2010). Anoctamin 10 may have a role in regulation of compartmentalized calcium signaling including release of calcium from intracellular stores (Benarroch 2017). Calcium signaling is important in proper function of Purkinje cells and abnormal signaling has been shown to be a pathophysiological mechanism in autosomal dominant ataxias (Benarroch 2017). Perhaps, *ANO10* mutations lead to Purkinje cell dysfunction, calcium triggered neurodegeneration or both (Benarroch 2017).

8 Conclusion

Cerebellar ataxia due to ion channel dysfunction or disruption is an evolving topic as much of what we know regarding expression or function of the channels is still being investigated or verified. Combined with this improved knowledge and improved methods of identification and recognition with innovations such as whole exome sequencing, we may be able to use this information to tailor treatment to better ameliorate the effects of the loss or gain-of-function mutations affecting these channels.

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Part II
Biomarkers and Tools of Trials

How to Design a Therapeutic Trial in SCAs

Caterina Mariotti, Mario Fichera, and Lorenzo Nanetti

Abstract Spinocerebellar ataxias (SCAs) are rare autosomal dominant inherited neurological disorders characterized by progressive cerebellar symptoms. In the past decades, several pharmacological and non-pharmacological symptomatic treatments were tested in clinical trials for their efficacy towards ataxia, but no long-lasting effective therapies have been yet established.

In this chapter we briefly reviewed the literature on both pharmacological trials and rehabilitating treatments performed in SCAs, with the aim of gathering information on trial objectives and methodology and discuss fundamental elements to consider in future trials.

For the design of meaningful clinical trials, the research question and associated hypotheses need to be well understood in terms of characteristics of the disease, intervention under study, target population, and measurement instruments. Randomized placebo-controlled designs are considered the primary research methodology for control of biases and confounding variables. New adaptive trial designs are also providing interesting options in order to reduce the number of subjects and speed up therapeutic deployment in rare diseases. In fact, the most challenging factors in clinical trials for SCA diseases are to maximize trial power with the minimum number of subjects, and to rely on the most sensitive outcome measures. Large collaborative initiatives on natural history studies for SCAs will provide the perfect support to ensure recruitment of a correctly powered number of patients, the number of appropriate sample sizes with targeted selection of stratified patient groups, and the knowledge about responsiveness to changes of the currently available outcome measures.

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_8

At present, the most important gap to be filled with respect to trial readiness in SCAs is the definition of outcome measures that will efficiently capture disease-related functional and structural changes occurring during disease course or in response to therapeutical interventions. The rarity and additional clinical diversity of conditions may require differential outcome sets for different purposes and different phases of the diseases (e.g., pre-clinical phase, and symptomatic stages). The other necessary and complementary element to advance in cure of SCAs will be availability of innovative and efficacious therapeutic options allowing real improvements in patient's life.

Keywords Randomized controlled trials (RCT) · Outcome measure · Trial design · Clinical interventions · Trial phases

1 Introduction

Study designs are commonly classified in two main categories: studies in which the subjects are merely observed and their characteristics recorded for analysis, called observational studies, and studies involving an active intervention, such as a drug, a procedure, or a treatment, called experimental studies (Dawson-Saunders and Trapp 2004).

Observational studies include cases-series, case control, cross-sectional, and cohort studies. Cross-sectional studies analyze data of a group or groups of subjects collected at one time. Case-control and cohort studies involve an extended observational period and, thus, are longitudinal studies. Longitudinal observational studies are extremely important because they provide valuable data on the reliability and responsiveness to change of the utilized outcome measures, such as effect size, standardized response mean, and signal-to-noise ratio. These indexes allow an estimate of the "true" change, related to disease progression, compared to random variability of repeated assessments. Further, such datasets yield important information on the interrelation of outcome assessment at different levels which can, for example, help to estimate the clinical relevance of changes observed. Clinical trials are experimental studies involving humans, and their purpose is to draw conclusions about a particular procedure or treatment. There are two major categories of clinical trials: those with and those without controls.

Controlled trials are studies in which the experimental drug or procedure is compared with a group treated with another drug or procedure, a placebo, or a previously accepted treatment. The two most common designs are (a) non-randomized controlled trials (non-RCTs), and (b) randomized controlled trials (RCTs). In non-randomized controlled trials, a concurrent comparison group is part of the study, and patients are allocated to this group by a nonrandom process. Data from such studies are usually considered reliable only if confirmed by a randomized study or by a meta-analysis of a number of similarly designed nonrandomized studies.

In RCTs, individuals are randomly allocated to two or more treatment groups, which usually include a standard treatment group and one or more experimental groups (Stanley 2007). To reduce the chances that both treated subjects and the rater investigators may introduce a bias in the evaluations by favoring what they are expecting or hoping to see, the trial may be designed as a double-blind trial. In this case neither the subjects nor the investigators are aware whether the subject is assigned to experimental treatment or to control arm. Control may be a placebo, a sham procedure or the treatment or procedure commonly used, called standard of care or reference standard.

Clinical trials are also classified by study objective and phases, and the conventional model of progressing from phase I to phase III is considered the standard paradigm for drug development.

Phase I trials evaluate safety of a new drug or intervention (usually involving 10–30 subjects), phase II trials assess efficacy (20–50 patients), and phase III trials confirm safety and efficacy in a much larger group of subjects (usually 100–1000 patients). Phase III RCTs usually are and should be powered to confirm hypothesized effect of treatment, usually informed by effects of observed in phase II. According to their purposes, phase III trials are also called "confirmatory trials."

More recently, adaptive trial design has been proposed as a mean to increase the efficiency of RCT. An adaptive design is defined by the US Food and Drug Administration (FDA) as "a clinical trial design that allows for prospectively planned modifications to one or more aspects of the design based on accumulating data from subjects in the trial" (FDA 2019). The European Medicine Agency (EMA) defines a study design as adaptive "if the statistical methodology allows the modification of a design element (for example, sample-size, randomization ratio, number of treatment arms) at an interim analysis with full control of the type I error" (EMA 2007). Control for type I error in clinical trial represents the probability of identify a treatment effect, when in real the treatment has no effect (false positive). Adaptive designs are applicable to both exploratory and confirmatory clinical trials.

Adaptive designs for exploratory clinical trials deal mainly with finding safe and effective doses or with dose–response modeling (Bhatt and Mehta 2016). These trials allocate patients to multiple different treatment doses, and patient responses are assessed at interim analyses. The purpose of the adaptive design is to be able to allocate more patients to the treatment doses of interest, while reducing allocation of patients to non-informative doses or doses eliciting safety concerns. In general, adaptive design allows a modification in randomization ratio, treatment arms, sample size estimation, and trial hypothesis in response to interim analyses results (Bothwell et al. 2018).

Adaptive designs for confirmatory trials have been distinguished in different categories, such as, for example, seamless phase II–III designs, and sample-size re-estimation designs.

Seamless phase II–III design reduces the time lag between phase II and III and allows continuing the trial without stopping patient enrolment and, more importantly, advance at least part of the study population seamlessly into the next phase of the study. The two main advantages of adaptive seamless design are the reduction of numbers needed until confirmation of efficacy is reached and the possibility to

adapt design to observations if there remains uncertainty at study start, for example, on features of the population under study (Bhatt and Mehta 2016; Bothwell et al. 2018).

The final step, after successful confirmatory phase III studies, is to monitor the safety of an approved intervention in a "real world" scenario, which is the purpose of phase IV studies.

2 Lessons from Clinical Trials Performed in SCAs

Spinocerebellar ataxias (SCAs) are rare, clinically, and genetic heterogeneous disorders, transmitted with an autosomal dominant mode of inheritance. In the past decades, several interventional clinical trials have been performed in SCA patients (Salman 2018; Zesiewicz et al. 2018); however, no long-lasting symptomatic therapies or disease-modifying therapies have been identified so far.

Several pharmacological clinical trials were designed to test a number of compounds not specifically developed for SCA diseases, but previously approved for human administration with various medical indications. Compounds include antioxidant agents, neuroprotective factors, antiepileptic medications, or supplement (Yap et al. 2021). The majority of these compounds were proposed after encouraging observation of symptomatic effects reported in a limited number of subjects; however, none of the pharmacological approach investigated so far were confirmed as efficacious therapies for SCAs. Although the ideal goal will be a disease modifying effect, able to slow, or halt disease progression, the identification of compounds with a significant and long-lasting symptomatic effect can also serve as means of modifying clinical course. This is for example the case of the treatment with acetazolamide or 4-aminopyridine in dominant episodic ataxia type 2 (EA2). In these patients, in fact, the reduction in frequency and severity of the ataxia attacks can greatly alleviate clinical dysfunction and substantially modify patient experience.

In the following sections, we will briefly review the literature on clinical trials for both pharmacological and rehabilitating treatments performed in SCAs, with the aim of gathering information about trial methodologies and obtaining fundamental elements to consider in future trials.

2.1 Pharmacological Interventions

We briefly reviewed the literature on pharmacological interventional trials in SCAs performed during the last two decades to collect and analyze overall information about trial design, trial objectives, study duration, number and characteristics of enrolled subjects, and primary endpoint of the studies.

We analyzed 25 studies (from January 2001 to February 2022) (Table 1). All studies can be categorized as phase II trials, except the study reported by Nishizawa

Table 1 Comparison of study design, duration, and patient characteristics in recent pharmacological interventional trials in SCAs

Drug	Subjects N. Drug/ Placebo	Study design	Duration (weeks)	Center N.	Ataxia type	Outcome measure	Mean change End of study Drug	Mean change End of study Placebo	Reference
Acetyl-DL-leucine	13/0	OL case series	1	1	SCA1, SCA2, NGAs[a]	SARA	−3.3		Strupp et al. (2013)
Acetyl-DL-leucine	10/0	OL case series	1	1	SCA2, SCA6, SCA8, NGAs[a]	SARA	−0.6		Pelz et al. (2015)
3,4-diaminopyridine	15/0	OL	1	1	SCA6 and 16q-ADCA	ICARS	−0.6		Tsunemi et al. (2010)
Gabapentin	11/0	OL	4	1	SCA6	ICARS	−3.1		Nakamura et al. (2009)
Tandospirone	39/0	OL	4	1	SCA1, SCA2, SCA3, SCA6, NGAs[a]	ICARS	−3.2		Takei et al. (2010)
Fluoxetine	13/0	OL	6	1	SCA3	EDSS UPDRS	0.0 −2.0		Monte et al. (2003)
Acetazolamide	6/0	OL	88	1	SCA6	ICARS	ICARS score significantly reduced		Yabe et al. (2001)
IGF-1	13/0	OL	104	1	SCA3, SCA7	SARA	+0.6		Arpa et al. (2011)
Trehalose	14/0	OL multiple dose	26	1	SCA3	NESSCA SARA	No changes in ataxia scores		Zaltzman et al. (2020)
D-cycloserine	15	Single-blind, placebo lead-in phase	2	1	SCA3, SCA6, NGAs[a]	ICARS	−1.8	−0.6	Ogawa et al. (2003)

(continued)

Table 1 (continued)

Drug	Subjects N. Drug/ Placebo	Study design	Duration (weeks)	Center N.	Ataxia type	Outcome measure	Mean change End of study Drug	Mean change End of study Placebo	Reference
Branched-chain amino acid	16	RCT Crossover multiple doses	4	1	SCA6, SCA7, NGAs[a]	ICARS	−4.3[b]	−1.4	Mori et al. (2002)
Buspirone	20	RCT Crossover	12	1	SCA1, SCA2, SCA3, SCA6, FA, SCA17, DRPLA, NGAs[a]	ICARS	+1.6	+3.4	Assadi et al. (2007)
Trimethoprim-sulfamethoxazole	22	RCT Crossover	26	1	SCA3	Ataxia rating scale	−0.1	−0.6	Schulte et al. (2001)
Valproic acid	24/12	RCT multiple doses	12	2	SCA3	SARA	−2.6[b]	−0.8	Lei et al. (2016)
Ondansetron	23/23	RCT	1	4	SCAs, FA, NGAs[a]	ICARS	−5.1	−8.0	Bier et al. (2003)
Varenicline	9/9	RCT	8	2	SCA3	SARA	−1.9	−0.9	Zesiewicz et al. (2012)
Riluzole	20/20	RCT	8	1	SCA1, SCA2, SCA28, FRDA, FXTAS, NGAs[a]	ICARS	−7.1	+0.2	Ristori et al. (2010)
Riluzole	28/27	RCT	52	3	SCA1, SCA2, SCA6, SCA8, SCA10, FA	SARA	−1.0	+1.7	Romano et al. (2015)
Riluzole	22/23	RCT	52	8	SCA2	SARA	+0.5	+0.3	Coarelli et al. (2022)

Docosahexaenoic acid	5/5	RCT	16	1	SCA38	SARA ICARS	−3.0 −5.0	−0.3 +0.4	Manes et al. (2017)
Zinc sulphate	18/18	RCT	26	1	SCA2	SARA	SARA scores lowered without differences between treatment arms		Velázquez-Pérez et al. (2011a)
Lithium	31/31	RCT	48	1	SCA3	NESSCA SARA	−0.35 compared to placebo −0.96 compared to placebo		Saute et al. (2014)
Lithium	9/8	RCT	48	1	SCA2	SARA	+0.3	+0.8	Saccà et al. (2015)
Rovatirelin[c]	140/138[c]	RCT	28	86	SCA6, SCA3, NGAs[a]	SARA	−1.6	−1.0	Nishizawa (2020)

Pharmacological interventional trials in SCAs performed between 2001 and 2022. Study characteristics (intervention, design, duration, included patients and number of centers involved) as well as main results are reported. Mean change refers to the mean difference in clinical scores between baseline and end-of-treatment. Negative figures indicate improvement in total clinical score

OL open-label, *RCT* Randomized Controlled Trial, *ICARS* International Cooperative Ataxia Rating Scale, *EDSS* Expanded Disability Status Score, *UPDRS* Unified Parkinson Disease Rating Scale, *SARA* Scale for the Assessment and Rating of Ataxia, *NESSCA* Neurological Examination Scale for Spinocerebellar Ataxias, *FA* Friedreich ataxia, *IGF* insuline growth Factor, *NGAs*[a] non-genetic ataxias, *wk* weeks, *n.a.* not available, [b]change for the most effective dosage, [c]pooled data from two studies

et al. (2020). In this phase III study, the authors reported a pooled retrospective analysis performed by combining the data from two phase II trials on rovatirelin. Thirteen studies recruited different populations of subjects including patients with different types of hereditary ataxia (SCAs and Friedreich ataxia) and patients with non-genetic ataxia, such as multisystem atrophy-cerebellar type.

We further identified 12 phase II studies with recruitment confined to single SCA genotypes: 6 studies recruited SCA3 patients (Schulte et al. 2001; Monte et al. 2003; Zesiewicz et al. 2012; Saute et al. 2014; Lei et al. 2016), 3 studies recruited SCA2 patients (Velázquez-Pérez et al. 2011a; Saccà et al. 2015; Coarelli et al. 2022), 2 studies SCA6 patients (Yabe et al. 2001; Nakamura et al. 2009), and 1 study was performed on SCA38 patients only (Manes et al. 2017).

An open-label design (OL) was adopted in 9 out of 25 studies (36%), one study was a single-blind study with a placebo lead-in phase, and 15 were randomized, placebo-controlled studies (60%). In OL trials, the mean number of enrolled subjects was 15 patients (median: 13, range: 6–39), the studies were all conducted in a single center, and the mean duration of the treatments was 26 weeks (median: 4 weeks, range: 1–88 weeks).

The 15 RCT studies had a mean of 50 enrolled patients (median: 36, range: 10–140), 7 were multi-center studies and 8 were single-center studies, and the mean duration of treatments was 24 weeks (median: 21, range: 1–52 weeks). Two studies had safety and tolerability as the main endpoint, and clinical effect of the treatments was considered secondary endpoint (Zaltzman et al. 2020; Saccà et al. 2015). In 18 out of 25, studies, endpoint and sample size calculation were not specifically provided. Only in five trials the primary endpoint was clearly stated, and it was represented by the evaluation of symptom improvement in treated patients versus no improvement/deterioration in placebo group (Saute et al. 2014; Romano et al. 2015; Nishizawa et al. 2020; Coarelli et al. 2022). The clinical outcome measures utilized in the trials were mainly the Scale for the Assessment and Rating of Ataxia (SARA) and the International Cooperative Ataxia Rating Scale (ICARS) (Schmitz-Hübsch et al. 2006; Trouillas et al. 1997).

At the end of the period of drug administration, clinical rating scores decreased in 20/25 studies (suggesting less severe ataxia signs), and increased in 5 studies. Differences between treatment and placebo arms were statistically significant in 5 out of 13 RCT studies, with a mean decrease of 2.2 points in SARA score, and 5.5 points in ICARS score (Mori et al. 2002; Ristori et al. 2010; Romano et al. 2015; Lei et al. 2016; Manes et al. 2017). Patients treated with rovatirelin showed a statistically significant improvement in SARA score, in comparison with placebo, only when pooled analyses of two trials were performed (Nishizawa et al. 2020). It is also worth to mention that in several RCTs (usually with less than 28-week duration) a decrease in clinical scores was also frequently reported in the placebo treated subjects (−8.0 to −0.6 points in ICARS; −1.0 to −0.3 in SARA), thus demonstrating the extent of the placebo effect (Table 1).

Three compounds were tested in more than one study: acetyl-DL-leucine was tested in two OL trials including ataxic patients with different genetic and non-genetic diagnoses; lithium was tested in SCA3 and in SCA2 patients in two different

RCTs, and riluzole was tested at the same dosage in three different RCTs (Table 1). The first riluzole trial showed an improvement in ICARS scores after 8 weeks of treatment in 20 subjects with acquired and genetic ataxias (Ristori et al. 2010); the second trial showed improvement of SARA scores after 1 year of treatment in 28 subjects with different hereditary ataxias (Romano et al. 2015); and the third trial showed no effect of a one-year treatment in 22 patients with SCA2 (Coarelli et al. 2022). The different results in the three riluzole trials, based on the same dose regimen, most likely depend on differences on trial designs including patient number, trial duration, outcome measures, monocentric versus multi-center setting, and, above all, patient selection.

In sum, the presentation of all previous evidence may serve as a reference of difficulties and errors encountered in symptomatic interventions. The design of such studies may not be an appropriate template for upcoming trials that are most likely aiming to delay progression or even manifestation.

2.2 Rehabilitation Interventions

Physiotherapy is often suggested to SCA patients to improve gait, balance, and coordination. In the past years, several studies have assessed the impact of physical therapy on ataxia severity with positive results. Type of rehabilitating treatments varies between studies and includes conventional coordination and balance training, multidisciplinary inpatient rehabilitation, cycling, treadmill, occupational therapy, and computer-assisted training. Similarly, the timing and intensity of the intervention display high variability across studies.

As an example of clinical studies on ataxia rehabilitation for ataxia symptoms, we selected six RCTs providing Class I and Class II evidence according to a recent systematic review (Yap et al. 2021), and an OL study for its longer follow-up period of 1 year (Ilg et al. 2010).

In RCTs, the control groups received no intervention (Rodríguez-Díaz et al. 2018; Tercero-Pérez et al. 2019; Miyai et al. 2012; Bunn et al. 2015), conventional physical therapy (Wang et al. 2018), or health education advices with exercises of upper limbs (Chang et al. 2015).

Interventions were of different types, such as in-patient neurorehabilitation combining physical and occupational therapy (Miyai et al. 2012), intensive conventional physiotherapy (Rodríguez-Díaz et al. 2018; Tercero-Pérez et al. 2019; Ilg et al. 2010), exergames-serious videogames (Wang et al. 2018), balance training with optokinetic stimuli (Bunn et al. 2015), and cycling (Chang et al. 2015). Intensity of the intervention ranged from 15 minutes to 6 hours per day during weekdays; duration of the intervention regimen ranged between 4 and 24 weeks (mean: 9 weeks, median: 4 weeks). In four studies, the trial enrolled a homogenous population of patients with the same genotype (Rodríguez-Díaz et al. 2018;Tercero-Pérez et al. 2019; Wang et al. 2018; Bunn et al. 2015), while in three studies a mixed population of patients with ataxia of different etiology was considered. Mean number of treated

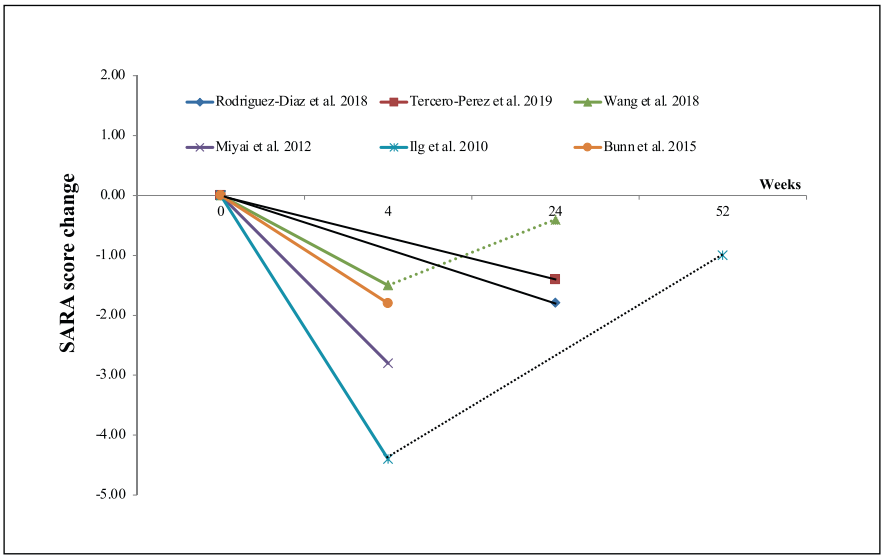

Fig. 1 Rehabilitation trials in SCA. The graph shows the mean changes in SARA score, from baseline to the end of rehabilitation period, in six recent interventional trials in SCA patients. Patients were treated with different types of rehabilitation and physical therapy. Decrease in SARA scores represent improvement in ataxia tasks. Solid line indicates treatment period, dashed line indicates follow-up period. SARA Scale for the Assessment and Rating of Ataxia

patients was 21 (median: 16 patients, range: 9–38). SARA scale was adopted as outcome measure for ataxia severity in all cases except one that used ICARS (Chang et al. 2015).

As displayed in Fig. 1, a significant improvement following rehabilitation was reported in the all studies, with a mean decrease in SARA score of 2.3 points. Data on long term follow-up after the intervention period were reported only in two studies (Wang et al. 2018; Ilg et al. 2010). The clinical assessments performed 24–52 weeks after the end of physiotherapy showed a progressive loss of the acquired benefit, suggesting that physiotherapy in SCA patients may be associated with a consistent but temporary effect on ataxic symptomatology.

3 Fundamental Aspects to Consider in SCA Clinical Trial

Information generated by trials is useful when the trial has clear hypotheses and research questions, and when the choice of study procedures and trial design are adequate to answer these questions.

The principal aspects that emerged from the revision of selected clinical trial in SCAs, both pharmacological and non-pharmacological, were the great variability in number of recruited subjects, trial duration, selection of SCA population, and choice

of outcome measures. These aspects greatly impact on results of the studies, and are fundamental aspects to be taken into consideration when planning clinical trials.

3.1 Participant Number and Trial Duration

The number of subjects is one of the greatest challenges to clinical trial due to the rarity of these disorders (Brooker et al. 2021). The global prevalence of SCAs assessed in population-based studies ranged from 0 to 5.6 cases per 100,000 individuals, with an average of 2.7 cases per 100,000 individuals (Ruano et al. 2014). The relative frequency of the different SCA subtypes shows marked geographical and ethnic variability, often owing to founder effects.

Currently, polyglutamine SCAs (SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA17, and DRPLA) are the most commonly recognized genetic forms of SCAs. As a matter of fact, polyglutamine SCAs are the easiest forms to be detected by diagnostic tests available in all ataxia centers, mainly because these forms share a common type of genetic mutation, located in a specific region of each causative gene. SCA3/MJD is the most common SCA worldwide (20–50% of families), followed by SCA2 (13–18%) and SCA6 (13–15%) (Klockgether et al. 2019). Other SCA subtypes caused by untranslated repeats or conventional mutations are rarer than polyQ SCAs, and their precise distribution and frequency may be underrated due to requirement of advanced molecular techniques for diagnosis.

All clinical trials for SCAs have the limiting factor of recruiting a sufficient number of patients, particularly in monocentric studies. The recruitment of a too small sample size could lead to inconclusive results or misleading results due to a selection bias. This is the case, for example, when a small group of enrolled patients may not be representative of the cohort of patients in the real-world setting.

There are at least two complementary approaches to increase trial power and therefore reduce the number of required subjects. One approach is to select the most appropriated and best performing outcome measures. The other approach is to choose a specific target group of patients, where lower variability and faster progression of the disease is expected. For both these aspects, a valuable contribution can derive from the existing collaborative efforts for natural history studies in SCAs such as the Integrated Project on Spinocerebellar Ataxia (EuroSCA) and Spinocerebellar Ataxia Type 3/Machado-Joseph Disease Initiative (ESMI) in Europe, and the Clinical Research Consortium for Spinocerebellar Ataxias (CRC-SCA) in the United States (Schmitz-Hubsch et al. 2010; Ashizawa et al. 2013; Jacobi et al. 2015; Lin et al. 2020).

In fact, Prospective cohort studies in SCAs provide fundamental information on clinical features, disease course, and on responsiveness to changes for specific SCA genotypes (Table 2). In addition, large collaborative research network can facilitate multicenter conduct of RCTs in SCA by faster recruitment of sufficient sample sizes.

It is necessary that the number of patients will be based on precise calculation of sample size that depends on primary hypothesis but is also closely linked to

Table 2 Mean annual change in natural history for the most common SCA genotypes

Cohort of patients Geographic site	N. of centers	Observation period (months)	Outcome measure	Subjects number per genotype	Annual change in ataxia scale scores	Reference
EUROSCA Multicenter European study	17	24	SARA	117 – SCA1 163 – SCA2 139 – SCA3	2.18 (0.17)[a] 1.40 (0.11)[a] 1.61 (0.12)[a]	Jacobi et al. (2011)
EUROSCA Multicenter European study	17	49 (median)	SARA	107 – SCA1 146 – SCA2 122 – SCA3 87 – SCA6	2.11 (0.12)[a] 1.49 (0.07)[a] 1.56 (0.08)[a] 0.80 (0.09)[a]	Jacobi et al. (2015)
CRC-SCA Multicenter USA study	12	Up to 24	SARA	39 – SCA1 52 – SCA2 93 – SCA3 54 – SCA6	1.61 (0.41)[a] 0.71 (0.31)[a] 0.65 (0.24)[a] 0.87 (0.28)[a]	Ashizawa et al. (2013)
Multicenter Study (France)	7	36	SARA	25 – SCA1 35 – SCA2 58 – SCA3 5 – SCA6 10 – SCA7	1.8 (0.3)[a] 1.3 (0.2)[a] 1.7 (0.2)[a] 0.4 (0.4)[a] 1.6 (0.4)[a]	Tezenas du Montcel et al. (2012)
Japan intractable diseases research	8	36	SARA	46 – SCA6	1.33 ± 1.40[b]	Yasui et al. (2014)
Single Center (Taiwan)	1	Up to 38	SARA	11 – SCA2 45 – SCA3 9 – SCA6 5 – SCA17	2.88 ± 2.32[b] 3.00 ± 1.52[b] 2.04 ± 0.76[b] 4.50 ± 2.22[b]	Lee et al. (2011)
Multicenter (Brazil)	2	12	SARA NESSCA	38 – SCA2	0.35–2.45[c] (SARA) 1.03–2.14[c] (NESSCA)	Monte et al. (2018)
Single Center(Brazil)	1	13	ICARS	34 – SCA3	5.1	França et al. (2009)
Single Center (Brazil)	1	60	NESSCA	105 – SCA3	1.26	Jardim et al. (2010)
Single Center (Taiwan)	1	60	SARA	10 – SCA1 37 – SCA2 118 – SCA3 25 – SCA6 9 – SCA17	1.23 1.52 1.60 0.99 3.26	Lin et al. (2019)

(continued)

Table 2 (continued)

Cohort of patients Geographic site	N. of centers	Observation period (months)	Outcome measure	Subjects number per genotype	Annual change in ataxia scale scores	Reference
Single Center (Cuba)	1	60	SARA	30 – SCA2	1.44	Rodríguez-Labrada et al. (2016)

For annual change of clinical scale scores, data are expressed as: [a]mean and standard error in parenthesis; [b]mean annual change ± standard deviation; [c]mean annual change in patients with <10 years disease duration and >10 year disease duration
ICARS International Cooperative Ataxia Rating Scale, *SARA* Scale for the Assessment and Rating of Ataxia, *NESSCA* Neurological Examination Scale for Spinocerebellar Ataxias

characteristics of the outcome chosen. Based on SARA score, the sample size estimation for a two-arm interventional study aiming at 50% reduction of progression and 80% power would require approximately 100 patients per group for a one-year trial (Jacobi et al. 2015). Highly reliable and sensitive outcome measures may allow shortening study duration and lowering its cost (Savelieff and Feldman 2021).

Trial duration needs to be carefully programmed. A short study duration is generally associated with a more pronounce placebo effect both in the treated patients and in placebo groups. The placebo effect in several SCA studies has been demonstrated to be more pronounced in trials lasting less than 24–28 weeks (Table 1). SCAs are slowly progressive neurodegenerative disorders, and a short period of observation may fail to detect a real pharmacological effect associated with a decrease or halting in the progression rate.

On the other hand, a too long study duration may be very expensive, and may imply that a patient population remains in a trial for prolonged period, also preventing the possibility of participating in other studies. This may result in competing priorities for different trials in which the same small number of subjects can participate.

When designing a clinical trial, the treatment duration has to be considered in order to be able to assess effective changes in either clinical severity and /or disease progression. The chances of capturing significant real changes greatly depend on (i) the effectiveness of study drug, (ii) sensitivity of outcome measures, and (iii) possibility of controlling for confounding variables.

A great number of natural history descriptions in different polyQ SCAs were able to demonstrate significant worsening over a one-year time period using different clinical rating scales. Indeed, the observation time (and sample size) needed for a trial is closely linked to reliability of the outcome chosen. Minimum requirement is that investigators can confidently expect a signal of progression/treatment above the "noise" of measurement, including the placebo effect. Thus, to determine appropriate trial duration for a study, investigators should search for evidence on outcome reliability and responsiveness, that is, expected progression rates over time from previous studies, definitions of smallest detectable change for the measure, and variability of change. It could be hypothesized that disease-modifying therapies may

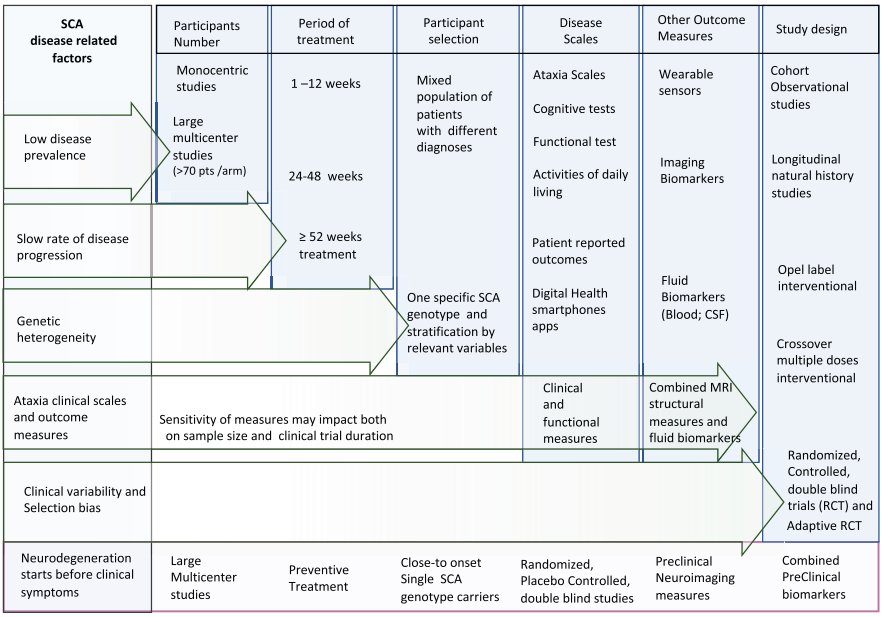

Fig. 2 Fundamental aspects to consider in SCA clinical trials. Scheme summarizing the critical aspects to consider in the design of clinical trials in spinocerebellar diseases

take longer to demonstrate a difference in disease progression compared to symptomatic therapies. In fact, a slowing or halting the natural course of SCAs may need several years to be ascertained, as minimal or no improvement in symptoms could be expected over time.

Considering the existing ataxia scales, and in particular of SARA scale, a 12-month observational period would be minimum time to detect an effect on progression either due to a sustained symptomatic improvement or to a true disease-modifying impact of the intervention (Fig. 2).

3.2 Selection of SCA Population

In selecting a population to enroll into a trial, researchers must consider the target use of the intervention because this will impact the possibility to generalize the results of the trial to the target population (Evans 2010). In trials testing symptomatic therapies different populations of ataxic patients were enrolled (Table 1) (Yap et al. 2021). Quite often both autosomal dominant and autosomal recessive forms were included in the same study, and, in a few trials, a mixed population of patients with genetic and non-genetic ataxias were also randomized in the same RCT (Romano et al. 2015). The enrollment of patients with different type of rare diseases may be advantageous to reach a larger number of subjects in a reduced period of

time; however, it would be difficult to draw appropriate conclusions, particularly when the observed differences in outcome measures between treated and control groups are relatively small. This type of approach has been proposed in particular for symptomatic treatments; however, patients with different SCA genotypes may have a different age at onset, different rate of disease progression, and different types of extracerebellar manifestations. For this reason, the enrollment of subjects with the same SCA genotype would allow a better control for confounding variables.

For trials on disease-modifying agents and even more so for trials on genotype-specific interventions, the approach of including patients with different SCA genotypes will be clearly inappropriate.

For example in the most frequent polyglutamine SCAs, the annual increase in SARA, a scale specifically developed for SCAs (Schmitz-Hübsch et al. 2006), was found to be different. Patients with SCA1 have the fastest rate of disease progression with annual SARA score increase of 2.1; in SCA2 patients there is an increase of 1.49; in SCA3 patients of 1.56, while SCA6 patients have the lowest rate of progression with an annual increase of 0.80 (Jacobi et al. 2015).

Furthermore to the core cerebellar symptoms, there is still significant variability in clinical presentations and progression of extra-cerebellar manifestations for SCA patients that may contribute in the overall assessment of the clinical status of the patients (Ashizawa et al. 2018). Each SCA genotype has a specific and characteristic pattern of non-ataxia signs that could influence progression and motor function.

The disease stage may also be a variable increasing heterogeneity in the same sample population. This would apply, for example, to variability in symptoms and rate of progression in very early or even pre-symptomatic stages of the disease, and in the late-disease stages when patients may be no longer ambulant. It has also to be considered that upcoming interventional trials would likely test not only symptomatic therapies, but rather disease-modifying therapies requiring matching the candidate drug to a specific patient population.

In this scenario, the time of intervention is also extremely important for the success of the therapies. In disease-modifying treatments, a preventive therapy may be more effective in the earliest stages of the disease or, ideally, even before the clinical manifestations. The same type of treatments may be not effective in reversing the disease process in symptomatic patients (Ashizawa et al. 2018). Taking into consideration the points discussed above, the advice for population selection for SCA clinical trial would be to ensure an adequate sample size of patients with the same genotype and with known confounders or predictors.

3.3 Outcome Measures

Clinical assessments of cerebellar disease symptoms are usually achieved by clinical rating scales.

Several expert centers and consortia have tested in the last decade different validated measures of structural and functional changes, quality of life, and disability

scales for natural history studies; however, the optimized and most suitable evaluations for clinical trials have not been yet completely established. SARA is the most widely used ataxia scale in SCAs has eight items that yield a total score of 0 (no ataxia) to 40 (most severe ataxia), assessing gait, stance, sitting, speech disturbance, finger chase, nose to finger test, fast alternating hand movements, and heel-shin slide. One of the advantages of SARA scale is that time for completion by a trained health care professional is less than 15 minutes in most cases (Schmitz-Hübsch et al. 2006; Perez-Lloret et al. 2021).

In SCAs, the sensitivity of the SARA scale and of other clinical outcome measures has been shown to vary in patients with different genotypes and disease stages. For SARA scale, no significant floor or ceiling effect for total score has been reported, both in validation and natural history studies on a mixed population of SCAs (Schmitz-Hübsch et al. 2006; Jacobi et al. 2015); however, a ceiling effect was later observed for long disease durations (Tanguy Melac et al. 2018).

Moreover, SARA scale is not adequate to assess the process of disease progression in pre-symptomatic subjects (Fig. 3). Disease-related changes may start before overt ataxia is visible and may continue in non-ambulatory patients, but these changes are not properly capture by the clinical scale alone.

New technological innovations are being investigated in order to remove any bias from a human rater, and to provide objective longitudinal data on patient symptoms. For example, the use of wearable sensors or smartphones to capture

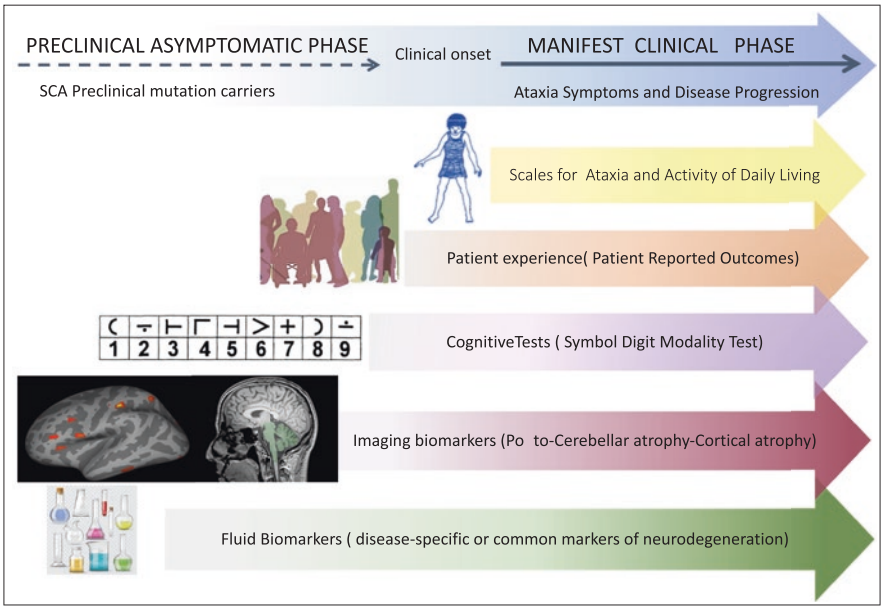

Fig. 3 Measures of disease progression in SCAs. Different measures of changes occurring during disease progression in spinocerebellar ataxias, from the pre-ataxic phase to the onset of manifest clinical symptoms

movements has been recently proposed for remote tracking of patients' movements, and secure a record of symptoms on a day-to-day basis (Brooker et al. 2021).

Recent findings suggest that magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) biomarkers will provide objective biological readouts of disease activity and progression (Reetz et al. 2013; Adanyeguh et al. 2018; Brooker et al. 2021) (Fig. 3). Structural voxel-based morphometry can detect and longitudinally track regional atrophy not only during the symptomatic phases of SCA diseases, but also during the presymptomatic period close to phenotypic conversion (Jacobi et al. 2020; Nigri et al. 2020, 2022). MRS has also been shown to be an efficient and non-invasive method to analyze regional metabolic differences in both presymptomatic and symptomatic SCA patients.

In addition, several investigations are currently testing the possibility that specific biomolecules or metabolites in blood or cerebrospinal fluid (CSF) may correlate with the disease progression. Some of these molecules are disease specific, as for example the mutant proteins of polyglutamine SCAs or other polyglutamine disorders such as Huntington disease. Other proposed biomolecules are common to several neurodegenerative disorders, as for example the neurofilament protein (Khalil et al. 2018).

In a recent phase I–IIa clinical trial for Huntington disease, the measurement of the disease-causing protein in CSF has been used as a surrogate exploratory endpoint to evaluate the possible effect of a therapy with antisense oligonucleotides (ASO) (Tabrizi et al. 2019). The observation of significantly lowered levels of mutant huntingtin in CSF, and the absence of serious side effects, prompted to the design of a large phase III study, with more than 800 patients enrolled worldwide. Unfortunately, a planned review of the data by an independent committee of experts led to the recommendation of an early termination of the trial, concluding that the drug's potential benefits did not outweigh its risks (Kwon 2021).

These results, though disappointing, showed the great importance of considering biomarkers as a useful tool in strict connection with clinical measures centered on patient medical conditions. Very important for study designs are also the patient-based reported outcome measures (PROMs). PROMs enable patients to report on their quality of life, daily functioning, symptoms, and may capture other aspects of their health and well-being (Black 2013). In a recent study in SCA3 patients, Maas and colleagues observed discordance between patient-reported and clinician-based outcomes indicating that these measures genuinely evaluate distinct aspects of disease and emphasize their complementariness in therapeutic trials (Maas et al. 2021).

The specific features of the currently available outcomes for SCAs have great implications for study design. It is possible that the combined use of both functional clinical scales and structural brain imaging data could improve sensitivity to changes in response to treatment and contribute to more efficient trial design in future trials. Continuous biomarker, like CSF wet biomarkers, atrophy from MRI scans, or quantitative measurement of ataxia should be preferred to clinical outcomes alone.

In addition, using repeated measures in the same individual or using continuous outcome variables may enhance statistical efficiency, depending on the properties of the outcome measures (Whicher et al. 2018). To increase compliance and acquire

more frequent evaluations, a hybrid trial model in which some study visits are done at home, some are carried out in the clinic, and some interactions take place remotely has been found to be preferred by patients and their accompanying family members.

3.4 Trial Designs

The main goal of clinical trials is to establish the authentic effect of an intervention separated from possible bias and confounding variables (Evans 2010). RCTs have long been considered the primary research study design because it may provide the best insurance that the results may be due to the intervention; however, to be really informative RCTs need to rely on clear endpoint and rigorous study procedures and sample size calculation.

An important aspect of RCT is randomization. Randomization is a crucial tool that helps control for bias in clinical trials favoring the balance between treatment and control with respect to participant characteristics (Evans 2010). In double-blind RCTs, the importance of a control group is highlighted by observation of a clear reduction in the ataxia clinical scores in placebo-treated subjects (Table 1). A reduction in clinical scores, thus indicating an improvement in ataxic symptomatology, is very likely a placebo effect since it is not associated with the natural history of SCA diseases, and is never reported in observational studies (Jacobi et al. 2015).

The inclusion of concurrent randomized controls is preferred over the use of historical controls. Historical controls are derived from previously conducted studies and are rarely used in clinical trials performed for drug development because they represent a nonrandomized population. In the absence of concurrent randomization, in fact, it is more likely the occurrence of selection bias with unknown influencing factors on score, progression, and variability that are unequally represented in the treatment arms.

Usually, the patient reported in historical control groups (as for example in natural history studies for poly-glutamine SCAs) had worse outcomes than patients participating in clinical trials in the control groups (see Table 1) (Dawson-Saunders and Trapp 2004). For this reason, the use of historical controls in interventional trials may support erroneous conclusions misleading often in favor of the tested therapies.

In addition, a well-design trial should consider stratification based on variables that are expected to impact on the observed outcome and thus also on the response to treatment.

In stratified randomization, separate randomization schedules should be prepared for each of the confounding variables. For example, in polyQ patients it would be important to ensure a correct balance in the treatment arms of several variables that can have an impact on the outcome, such as the baseline scores at the ataxia rating scale, the length of the pathological triplet expansions, and presence and severity of neurological symptoms.

The stratified assignment to participant groups ensures an equal number of subjects with low or high clinical scores in both arms of the trial, and that early onset or

late onset patients are equally distributed. The problem with stratified trial design is that the sample size has to be large enough in order to be able to enroll an adequate number of patients for each treatment and for each stratum that will be subsequently analyzed.

Adaptive trial design represents a more recent strategy that can be applied to RCT. Adaptive trial designs may provide interesting options in a rare condition in order to reduce the number of subjects and speed up therapeutic deployment (Bhatt and Mehta 2016). In adaptive study designs, the sample size may be reduced by including multiple treatment options in a factorial study, in which two (or more) treatment comparisons are carried out simultaneously and compared with a unique placebo-controlled group. The design of seamless confirmatory randomized phase II/III studies could allow fewer patients and an overall shorter duration in respect to traditional RCT accomplishing phase II and phase III as separated studies. Other form of adaptive trial designs is being considered also for rare neurological diseases as a way to speed up the process for drug approval. Aim of these designs is to improve efficiency and to standardize procedures in the development and evaluation of different interventions under a common infrastructure. These new designs can be classified into "basket trials," "umbrella trials," and "platform trials" (Park et al. 2019). In basket trials, a targeted therapy is evaluated on multiple diseases that have common genetic or molecular background; this type of design may prove useful, for example, to assess simultaneously the same compound in different polyQ SCAs. Conversely, umbrella trials evaluate multiple therapies for a single disease, stratified according to molecular alteration or disease biomarker. Finally, platform trials can be described as multi-arm, multi-stage trials (Adaptive Platform Trials Coalition 2019). These trials aim to evaluate several interventions compared to a common control group, can be used continuously, and can be, hypothetically, perpetual. This design allows pre-specified adaptation rules for dropping ineffective treatments and for adding new intervention during the trial. Platform trials seem particularly suited when the population that can be enrolled is small, as in rare diseases.

3.5 Clinical Trial in Preclinical Stage SCA

In inherited adult-onset neurodegenerative disorders, such as SCAs, the possibility of predictive testing in at-risk family members allows the identification of mutation carriers several years or even decades before the manifestation of clinical symptoms (pre-symptomatic phase).

The definition of "pre-symptomatic" stage implies an objective criterion or threshold for the first recognition of ataxia symptoms to be used for inclusion criteria in clinical trials. It has been proposed the use of SARA scale, the validation of which indicated that a score of 3 or more differentiates controls from SCA patients with manifest ataxia (Schmitz-Hübsch et al. 2006). However, carriers of SCA mutation may present neurological, neuroimaging, or neurophysiological signs, indicating neurodegeneration, before any ataxia symptoms could be identified (Fig. 3). For

this reason, the term pre-clinical stage has been preferred for the disease period in which no symptoms are observed but paraclinical tests may reveal the presence of neuropathological processes (Maas et al. 2015). SCA mutation carriers not presenting either clinical or paraclinical abnormalities could be identified as asymptomatic carriers and this condition will start from birth until the observation of paraclinical abnormalities. Large multicenter studies on preclinical polyQ SCAs mutation carriers have provided the bases for the creation of mathematical models enabling the prediction of age of onset from the length of CAG repeat region, and in some model, from the age of onset of the affected parents (Globas et al. 2008; Velázquez-Pérez et al. 2011b; Tezenas du Montcel et al. 2014). The estimated age of onset could represent a valuable parameter in order to enroll subjects having homogeneous characteristics in respect to expected ataxia manifestations. A critical issue for the design of clinical trials in preclinical subjects is the absence of validate outcomes that efficiently and rapidly could measure disease progression and reflect the effects of an intervention. Presently, the more promising tools are represented by MRI structural analyses of specific areas of atrophy, such as in cerebellum and brainstem, that are already identified in preclinical phase and progress over time (Reetz et al. 2018; Nigri et al. 2020, 2022).

In addition, neurofilament light (NfL) represents a valuable biomarker of neurodegeneration in polyQ SCAs. Blood levels of NfL have been found increased at the ataxic stage and already at the presymptomatic stage, as compared with healthy controls (Wilke et al. 2018, 2022; Peng et al. 2020; Yan et al. 2021; Coarelli et al. 2021). In the presymptomatic stage, the NfL increases with proximity to the expected onset, being associated with early neurodegeneration, and even predicting cerebellar volumetric changes (Coarelli et al. 2021; Peng et al. 2022). For trials in carriers, NfL levels could help in stratifying the subjects on the basis of their proximity to disease onset, and could be used to monitor possible changes occurring in response to treatment (Coarelli et al. 2021; Wilke et al. 2022).

In last years, regulatory agencies, namely EMA and FDA (EMA 2014; FDA 2013), have already considered the possibility of designing trials to treat subjects at risk of developing neurodegenerative diseases, in particular Alzheimer disease and other dementias. However, there are fundamental issue that need to be further addressed, as, for example, when to start a potentially disease modifying treatment, how many years a clinical trial should last, how to correctly calculate sample size. Future trials testing gene therapy for specific SCA genotypes might certainly require patients at an early stage of disease, including preclinical subjects. Treating neurodegenerative conditions before overt symptoms onset may be more effective in limiting the progression of the neurodegenerative process and, therefore, have a more pronounced effect in ameliorating patient quality of life and life-expectancy (Ashizawa et al. 2018).

4 Conclusions

Undoubtedly, inherited cerebellar ataxias need the development of efficacious therapeutic approaches both symptomatic and disease-modifying. The prompt demonstration of the effectiveness of new interventions needs the design of efficient clinical trials.

One obstacle to develop precise treatments for SCAs is the diversity of causes of the condition. Mutations in more than 40 genes can result in SCA and there are likely hundreds of disease-causing mutations (Bushart et al. 2016).

The over 20-year experience in pharmacological and nonpharmacological intervention in patients with SCAs had led to important acquisitions and understanding on how to approach clinical trial design.

In addition, international disease registries provided accurate knowledge of the natural history for the most frequent SCA genotypes, and will certainly play a fundamental role in assisting and facilitating enrolment in future multi-center trials.

The most important gap to be filled for the implementation of successful trials in SCA remains the availability of reliable and sensitive outcome measures. It is likely that none of the currently used clinical scales for ataxia will be sufficient as a single tool. It seems very plausible that the appropriate combination of neuroimaging structural data, peripheral biomarkers, digital measures, and patient reported outcome could represent more valuable instruments to follow disease progression and response to therapies in interventional trials.

Upcoming clinical trials most likely will focus on single ataxia genotype and the possible intervention will be specific for the causative genetic defect, allowing clinically relevant and clearly recognizable improvements in patient's life.

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Therapy Development for Spinocerebellar Ataxia: Rating Scales and Biomarkers

Chih-Chun Lin and Sheng-Han Kuo

Abstract Spinocerebellar ataxias (SCAs) are a group of dominantly inherited disorders with progressive cerebellar dysfunction. Although there are no Food and Drug Administration-approved therapies in the United States for SCAs, the efforts in the past decades have helped us gain an understanding of the pathomechanisms and disease progression, especially with cytosine-adenine-guanine (CAG) expansion SCAs. This has set the stage for the development of symptomatic or disease-modifying therapies. However, when designing clinical trials, it is important to choose suitable clinical rating scales to monitor disease progression and response to therapeutic interventions. In addition, studies need to incorporate appropriate biomarkers that can be used to test for target engagement. This chapter will review the rating scales and recent advances of biomarkers, focusing on CAG-repeat SCAs. Understanding these available tools will facilitate the design of clinical trials to find therapies for SCAs.

Keywords Rating scales · Biomarkers · Physiology · Imaging

1 Introduction

CAG-repeat spinocerebellar ataxias (SCAs) include SCA1, 2, 3, 6, 7, and 17, are most common among the 48 subtypes of SCAs, and their clinical progression has been characterized in the natural history studies in the United States (Ashizawa et al. 2013), Europe (Jacobi et al. 2011), Japan (Sasaki et al. 1996; Yasui et al. 2014),

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_9

Brazil (Franca Jr. et al. 2009; Rezende et al. 2018; Piccinin et al. 2020), Portugal (Mendonca et al. 2018), Taiwan (Lee et al. 2011), and China (Guo et al. 2020). The well-studied disease progression and high disease penetrance make CAG-repeat SCAs good candidates for clinical trials studying gene therapies or anti-sense oligonucleotides (ASOs).

All clinical trials for SCAs have an inherent challenge in recruiting a sufficient number of patients, owing to its rarity with a collective prevalence of 1–6 per 100,000 (Ashizawa et al. 2018). Furthermore, in addition to the core cerebellar symptoms, there is still significant variability in clinical presentations of extracerebellar manifestations for SCA patients. To solve these challenges, several international collaborations and consortiums have been set up to study the natural history and diverse clinical features of SCAs with validated clinical rating scales. Most of the rating scales for ataxia evaluate various neurological symptoms in different body parts to gauge the ataxia severity. Repeated assessment of the ataxia severity provides information on disease progression in natural history studies and responsiveness to therapy in clinical trials. In addition, there are rating scales to measure the non-ataxic symptoms and non-motor features of SCAs. Finally, rating scales are developed to assess the functional status, including activities of daily living of SCA patients.

Other than rating scales as endpoints for clinical trials, biomarkers are also key to trial success. The development of biomarkers includes three aspects: neuroimaging, fluid, and physiology (Fig. 1). Each biomarker serves a unique purpose to provide an objective measurement of a specific aspect of the disease. Among these, biomarkers for tracking disease progression and testing for target engagement are the main focus of research. This chapter will review the existing clinical rating scales and recent developments in biomarkers for SCAs.

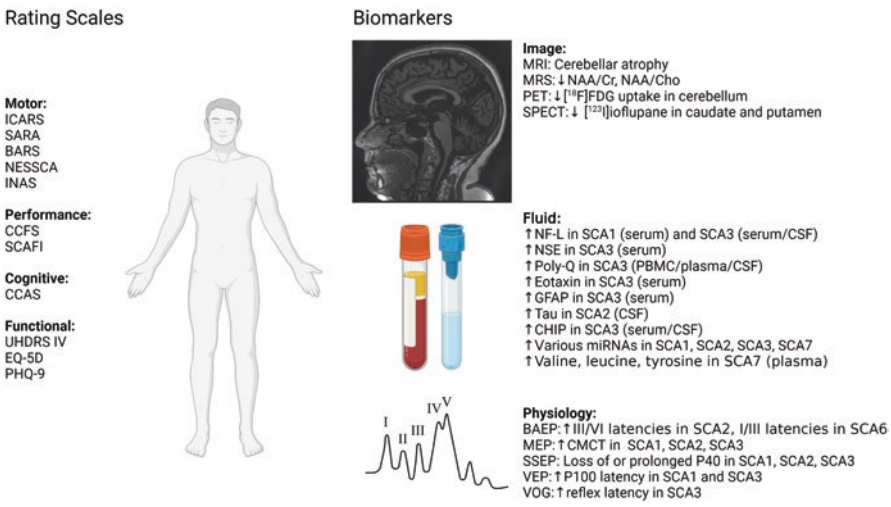

Fig. 1 The summary of biomarkers of SCAs, including clinical rating scales, neuroimaging, and biofluid biomarkers. NF-L: neurofilament light chain. See text for other abbreviations

2 Rating Scales (Table 1)

Most of the clinical rating scales for SCAs focus on assessing the cerebellar dysfunction to measure the disease severity, which is often the primary endpoint for clinical trials for SCAs. However, some SCAs present with neurological symptoms other than ataxia. Therefore, rating scales capturing the full motor symptoms of

Table 1 Rating scales for spinocerebellar ataxias

Rating scale	Key features (score range)	Reference
Motor		
International Cooperative Ataxia Rating Scale (ICARS)	Evaluate ataxia motor symptoms, including oculomotor examination (0–100)	Trouillas et al. (1997)
Scale for the assessment and rating of ataxia (SARA)	Most extensively adopted scale for ataxia with 8 domains assessed but no oculomotor evaluation (0–40)	Schmitz-Hubsch et al. (2006)
Brief Ataxia Rating Scale (BARS)	A concise 5-domain scale, including oculomotor evaluation (0–30)	Schmahmann et al. (2009)
Neurological Examination Score for the Assessment of Spinocerebellar Ataxia (NESSCA)	Originally developed for SCA3 but later validated in SCA2; includes assessments for cerebellar and extra-cerebellar domains: neuropathy, parkinsonism, and pyramidal signs (0–40)	Kieling et al. (2008) and Monte et al. (2017)
The Inventory of Non-Ataxia Symptoms (INAS)	Evaluate extra-cerebellar symptoms associated with SCA patients; part of the scale is subjective, patient reported outcomes (0–16).	Jacobi et al. (2013a)
Performance		
Composite Cerebellar Functional Severity Score (CCFS)	Consists of two performance-based tasks: 9-hole peg test and click test	du Montcel et al. (2008)
SCA Functional Index (SCAFI)	Consists of three functional measures: timed 8-m walk, 9-hole peg test, and PATA repetition	Schmitz-Hubsch et al. (2008b)
Cognitive		
Cerebellar Cognitive Affective Syndrome Scale (CCAS)	Assess the cognitive function of ataxia patients	Hoche et al. (2018)
Functional		
UHDRS IV	Patient reported functional capacity	Unified Huntington's Disease Rating Scale (1996)
EQ-5D	Patient reported functional capacity and overall health	du Montcel et al. (2008)
Patient Health Questionnaire-9 (PHQ-9)	A self-administered questionnaire to assess the severity of depression	Kroenke et al. (2001)

Modified from Chen et al. (2021)

SCAs are also necessary. Finally, it is vital to have rating scales track cognitive impairment associated with cerebellar dysfunction. We will discuss each in detail.

2.1 Scales for Motor Dysfunction

International Cooperative Ataxia Rating Scale (ICARS) is the first rating scale developed for ataxia, introduced in 1997 by the Ataxia Neuropharmacology Committee of the World Federation of Neurology (Trouillas et al. 1997). ICARS measures the severity of ataxia with 19 items, a total score of 100 divided into 4 subscales: posture and gait disturbances, limb ataxia (kinetic functions), dysarthria (speech disturbances), and oculomotor disorders (Trouillas et al. 1997). However, there is redundancy in the subscales, leading to the development of more concise rating scales, such as the Scale for the Assessment and Rating of Ataxia (SARA) (Schmitz-Hubsch et al. 2006). SARA comprises 8 rating items (gait, stance, sitting, speech, finger chase, nose-finger test, fast alternating hand movements, and heel-shin slide) with a total score of 40. However, SARA does not include an item to assess ocular abnormalities. To balance the limitations of ICARS and SARA, the Brief Ataxia Rating Scale (BARS) was derived from a modified version of ICARS. BARS consists of only five items: gait, kinetic function of legs and arms, speech, and eye movements, with a total score of 30 (Schmahmann et al. 2009). BARS also has more levels for each item compared with ICARS.

Although cerebellar symptoms remain the hallmark of SCAs, it is common to find neurological symptoms outside the cerebellar domain. The Neurological Examination Score for the Assessment of Spinocerebellar Ataxia (NESSCA) was initially developed to assess individuals with SCA3 (Kieling et al. 2008) and later validated in SCA2 (Monte et al. 2017). NESSCA assesses not only ataxia symptoms but also non-ataxia motor symptoms: eyelid retraction, fasciculations, sensory loss, blepharospasm, rigidity, bradykinesia, distal amyotrophy, sphincter dysfunction, vertigo, and optic atrophy. The Inventory of Non-Ataxia Symptoms (INAS), on the other hand, focuses mainly on the non-cerebellar neurological signs, such as spasticity, fasciculations, myoclonus, tremor, dystonia, and vibratory sense. Oculomotor abnormalities, such as nystagmus and hypo- or hyper-metric saccades, are also included, which can also be the result of cerebellar pathology (Jacobi et al. 2013a). Including INAS in a clinical trial is helpful in monitoring the progression of the non-ataxia motor symptoms in SCA patients.

Among these rating scales, SARA has become the most adopted rating scale in clinical studies for SCAs to assess the core cerebellar symptoms since its introduction in 2006. SARA has been extensively validated with an excellent inter-rater reliability (interclass coefficient = 0.98) and test-retest reliability (interclass coefficient = 0.90) (Schmitz-Hubsch et al. 2006). Importantly, the natural history studies of SCA1, 2, 3, and 6 in the cohorts in Europe and the United States adopted SARA as the primary rating scale (Ashizawa et al. 2013; Jacobi et al. 2011; Diallo et al. 2018; Moriarty et al. 2016; Jacobi et al. 2015; Schmitz-Hubsch et al. 2008a),

demonstrating SARA scores progress linearly in these SCAs. However, the rates of progression differ among different types of SCA, as demonstrated by both SARA and INAS in patients with SCA1, 2, 3, 6, and 17 (Ashizawa et al. 2013; Jacobi et al. 2011; Yasui et al. 2014; Piccinin et al. 2020; Lee et al. 2011) (Table 2). There also appears to be a geographical difference in the rate of progression measured by SARA, even within the same type of SCA (Table 2).

Recently, SARA^{home}, a video-based scale modified from SARA, was designed to assess ataxia severity at home to capture day-to-day and within-day fluctuations (Grobe-Einsler et al. 2021). SARA^{home} includes 5 items from SARA (gait, stance, speech, nose-finger test, and fast alternating hand movements) with scores ranging from 0 to 28. To validate SARA^{home}, SARA scores, measured in neurology clinics, were compared to SARA^{home}, captured by videos. The scores for SARA^{home} correlated highly and progressed in parallel with the total SARA scores (Grobe-Einsler et al. 2021). In addition, SARA^{home} also demonstrated its ability to capture the variability of ataxia severity over the period of two weeks, allowing repeated measures for more accurate ataxia assessment (Grobe-Einsler et al. 2021).

Table 2 Rate of progression measured by SARA and INAS

Diagnosis	The annual SARA increment	The annual INAS increment	Location
SCA1	1.61 ± 0.41 (Ashizawa et al. 2013) 2.18 ± 0.17 (Jacobi et al. 2011)	0.56 ± 0.11 (Jacobi et al. 2011)	United States (Ashizawa et al. 2013) Europe (Jacobi et al. 2011)
SCA2	0.71 ± 0.31 (Ashizawa et al. 2013) 1.40 ± 0.11 (Jacobi et al. 2011) 2.88 ± 2.32 (Lee et al. 2011)	0.30 ± 0.08 (Jacobi et al. 2011)	United States (Ashizawa et al. 2013) Europe (Jacobi et al. 2011) Taiwan (Lee et al. 2011)
SCA3	0.65 ± 0.24 (Ashizawa et al. 2013) 1.61 ± 0.12 (Jacobi et al. 2011) 0.71 (Piccinin et al. 2020) 3.00 ± 1.52 (Lee et al. 2011)	0.30 ± 0.08 (Jacobi et al. 2011)	United States (Ashizawa et al. 2013) Europe (Jacobi et al. 2011) Brazil (Piccinin et al. 2020) Taiwan (Lee et al. 2011)
SCA6	0.87 ± 0.28 (Ashizawa et al. 2013) 0.35 ± 0.34 for the first year (Jacobi et al. 2011) 1.44 ± 0.34 for the second year (Jacobi et al. 2011) 1.33 ± 1.40 (Yasui et al. 2014) 2.04 ± 0.76 (Lee et al. 2011)	0.10 ± 0.08 (Jacobi et al. 2011)	United States (Ashizawa et al. 2013) Europe (Jacobi et al. 2011) Japan (Yasui et al. 2014) Taiwan (Lee et al. 2011)
SCA17	4.50 ± 2.22 (Lee et al. 2011)		Taiwan (Lee et al. 2011)

Modified from Chen et al. (2021)
Values are given as mean ± SE
INAS The Inventory of Non-Ataxia Symptoms, *SARA* scale for the assessment and rating of ataxia

2.2 Scales for Performance

The items in the rating scales mentioned above typically evaluate an isolated neurological function, such as a specific cerebellar function or the strength of a specific muscle group. However, each daily activity encountered may require a combination of several neurological functions. To better assess the functional performance of SCA patients, two performance-based rating scales are commonly used, the Composite Cerebellar Functional Severity Score (CCFS) (du Montcel et al. 2008) and the SCA Functional Index (SCAFI) (Schmitz-Hubsch et al. 2008b). CCFS includes the 9-peg board test and the click test. The former measures the time for a patient to place 9 pegs into holes, and the latter measures how fast a patient can press two buttons alternatively for 10 times. CCFS measures the severity of appendicular ataxia but only the upper extremities. SCAFI extends the measurement to the lower extremities by adding a timed 8-m walk to assess the combination of lower limb function and balance. However, this test is only applicable to patients who can still ambulate, whether assistive devices were used. Furthermore, the use of different assistive devices cannot be accounted for in data analysis. An important feature for CCFS is that age should be considered in these performance tests since older healthy controls generally performed worse than the younger healthy controls (du Montcel et al. 2008). Therefore, age adjustment is needed. Another interesting point is that CCFS does not appear to be influenced by depressed mood (du Montcel et al. 2008), which is common among SCA patients (Lo et al. 2016). In summary, both CCFS and SCAFI demonstrate the real-world performance of SCA patients in activities involving coordination.

2.3 Scales for Non-motor Symptoms

The cerebellum projects extensively to various areas of the cerebral cortex to modulate cerebral function. As a result, dysfunction of the cerebellum can lead to a variety of cognitive symptoms in addition to motor impairments in patients with SCAs. To evaluate the cognitive dysfunction in patients with cerebellar ataxia, the Cerebellar Cognitive Affective Syndrome Scale (CCAS) assesses several domains of cognitive function (semantic fluency, phonemic fluency, category switching, verbal registration, digit span, cube drawing/copying, recalls, similarities, go-no-go, and affect) (Hoche et al. 2018). Monitoring cognitive dysfunction is particularly important, because impairments of these cognitive functions can significantly impact the quality of life in SCA patients.

Depression has been frequently reported in SCA3 (Kawai et al. 2004; McMurtray et al. 2006; Braga-Neto et al. 2012) and is one of the most commonly identified non-motor symptoms in patients with SCAs (Schmitz-Hubsch et al. 2011). The clinical studies in both Europe (Schmitz-Hubsch et al. 2011) and the United States (Lo et al. 2016), such as the EUROSCA and CRC-SCA natural history study (Lo et al. 2016;

Schmitz-Hubsch et al. 2011), adopted the Patient Health Questionnaire-9 (PHQ-9), a self-administered questionnaire, to assess the severity of depression (Kroenke et al. 2001). This is a 9-item questionnaire, with each item having four levels of scores with increasing severity (0–3), hence a maximal total score of 27. Mild, moderate, moderately severe, and severe depression correspond to 5, 10, 15, and 20 points (Kroenke et al. 2001).

2.4 Scales for Functional Capacity and Quality of Life

Ataxia researchers frequently measure the functional status of a patient with the following two scales, the Part IV of the Unified Huntington's Disease Rating Scale (UHDRS IV) and the EQ-5D. UHDRS IV measures the functional capacity with 25 questions to document the patient's capabilities in activities of daily living, handling financial matters, and performing at work (Unified Huntington's Disease Rating Scale 1996). EQ-5D measures both the functional level and the overall health of a patient (du Montcel et al. 2008). The version commonly used is EQ-5D-3L, which includes five 3-level questions to assess the patient's mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. An additional self-reported score between 0 and 100 reflects the patient's overall health state (Hurst et al. 1997). UHDRS IV and EQ-5D are important because they may reflect the response to treatment from the patient's perspective. In fact, there have been doubts that even if an improvement was found by rating scales based on neurological examinations, such as SARA, the patient might not find a perceivable change in his/her day-to-day experience. As a result, the inclusion of outcome measurements that reflect functional improvements reported by patients has been requested by the Food and Drug Administration in the United States in the design of clinical trials (Health USDo, Human Services FDACfDE, Research et al. 2006; Mercieca-Bebber et al. 2018).

3 Biomarkers

3.1 Neuroimaging Biomarkers

Neuroimaging has been one of the most studied modalities in the search for biomarkers for SCAs, because techniques such as magnetic resonance imaging (MRI) can detect atrophy of the cerebellum, a common finding in SCAs as the result of cerebellar degeneration. In addition, metabolic alterations of the cerebellum and related brainstem areas can be identified by positron emission tomography (PET) or magnetic resonance spectroscopy (MRS), often preceding structural changes. Single-photon emission computed tomography (SPECT) can assess the reserve of dopaminergic and GABAergic neurotransmission and cerebral perfusion. The neuroimage findings are summarized in Table 3.

Table 3 Major image findings

Modality	Major findings
MRI	*SCA1* ↓cerebellum, brainstem (Guerrini et al. 2004; Schulz et al. 2010), caudate (Schulz et al. 2010), putamen (Schulz et al. 2010), and temporal lobe (Schulz et al. 2010) ↓WM in cerebellar hemispheres (Goel et al. 2011) ↓Spinal cord (Martins Jr. et al. 2017)
	SCA2 ↓cerebellum (Guerrini et al. 2004; Reetz et al. 2018; Goel et al. 2011), brainstem (Guerrini et al. 2004; Reetz et al. 2018) ↓fractional anisotropy and mode of anisotropy in the brain stem, cerebellar peduncles, cerebellum, cerebral hemisphere WM, corpus callosum, and thalami (Mascalchi et al. 2015)
	SCA3 ↓cerebellum (Eichler et al. 2011; Etchebehere et al. 2001; Reetz et al. 2013; Schulz et al. 2010), deep cerebellar nuclei (Stefanescu et al. 2015), brainstem (Eichler et al. 2011; Reetz et al. 2013; Schulz et al. 2010), spinal cord (Faber et al. 2021; Fahl et al. 2015), basal ganglia (Reetz et al. 2013; Schulz et al. 2010), and temporal lobe (Schulz et al. 2010) ↓WM in cerebellum (Guimaraes et al. 2013), cerebellar hemispheres (Kang et al. 2014), brainstem (Guimaraes et al. 2013), bilateral thalamus (Kang et al. 2014) ↓fractional anisotropy in cerebellum (Guimaraes et al. 2013), brainstem (Guimaraes et al. 2013)
	SCA6 ↓cerebellum (Stefanescu et al. 2015; Eichler et al. 2011; Reetz et al. 2013; Schulz et al. 2010), deep cerebellar nuclei (Stefanescu et al. 2015), brainstem (Eichler et al. 2011; Reetz et al. 2013; Schulz et al. 2010), basal ganglia (Reetz et al. 2013)
	SCA17 ↓cerebellum (Brockmann et al. 2012; Reetz et al. 2010), caudate nucleus (Brockmann et al. 2012), limbic system and parietal precuneus (Reetz et al. 2010)
MRS	*SCA1* ↓Glu, NAA, NAAG, tNAA, Cho/Cr, Glu/Gln, NAA/Cho, NAA/Cr (Guerrini et al. 2004; Lirng et al. 2012; Oz et al. 2010, 2011; Joers et al. 2018; Doss et al. 2014; Lirng et al. 2012) ↑Glc, Gln, mI, Tau, tCr, Glc+Tau (Oz et al. 2010; Oz et al. 2011; Joers et al. 2018)
	SCA2 ↓Cho, Glu, NAA, tNAA, Cho/Cr, NAA/Cho, NAA/Cr (Guerrini et al. 2004; Lirng et al. 2012; Viau et al. 2005; Oz et al. 2011; Wang et al. 2012) ↑Gln, GSH, mI, Tau, tCr, Glc+Tau, mI/Cr (Viau et al. 2005; Oz et al. 2011; Joers et al. 2018)
	SCA3 ↓NAA, NAAG, tNAA, NAA/Cho, NAA/Cr (Joers et al. 2018; Wang et al. 2012; Huang et al. 2017) ↑mI, Tau, tCr, Glc+Tau (Joers et al. 2018)
	SCA6 ↓GABA, NAA, tNAA, NAA/Cho, NAA/Cr (Joers et al. 2018) ↑Lac mI, Glc+Tau (Oz et al. 2011; Joers et al. 2018)
	SCA17 ↓NAA/Cho, NAA/Cr (Lirng et al. 2012)
fMRI	↓cerebellar cortex and deep cerebellar nuclei (Stefanescu et al. 2015)

(continued)

Table 3 (continued)

Modality	Major findings
PET	*SCA1* [^{18}F]FDG:↓metabolism in cerebellum (Wullner et al. 2005), brainstem (Gilman et al. 1996; Wullner et al. 2005), cerebral cortex, caudate nucleus, putamen, thalamus (Gilman et al. 1996)
	SCA2 [^{18}F]FDG:↓metabolism in cerebellum (Wang et al. 2007; Wullner et al. 2005; Oh et al. 2017), brainstem (Wang et al. 2007; Wullner et al. 2005), parietal cortex (Wullner et al. 2005), parahippocampal gyrus (Wang et al. 2007), frontal cortex (Wang et al. 2007) [^{11}C]dMP:↓Dopamine transporter levels in putamen and caudate nucleus (Wullner et al. 2005)
	SCA3 [^{18}F]FDG:↓metabolism in cerebellum (Wang et al. 2007; Wullner et al. 2005; Soong et al. 1997; Soong and Liu 1998), brainstem (Wullner et al. 2005; Soong et al. 1997; Soong and Liu 1998), occipital cortex (Soong et al. 1997; Soong and Liu 1998), basal ganglia (Wang et al. 2007; Wullner et al. 2005), thalamus (Wullner et al. 2005), parahippocampal gyrus (Wang et al. 2007),↑metabolism in parietal and temporal cortices preclinically (Soong and Liu 1998) [^{11}C]dMP:↓Dopamine transporter levels in basal ganglia (Wullner et al. 2005) [^{11}C]MP4P:↓thalamus (Hirano et al. 2008)
	SCA6 [^{18}F]FDG:↓metabolism in cerebellum (Wang et al. 2007; Wullner et al. 2005; Oh et al. 2017; Soong et al. 2001), brainstem (Soong et al. 2001), basal ganglia (Wullner et al. 2005; Soong et al. 2001), cerebral cortex (Wang et al. 2007; Soong et al. 2001);↑temporal cortex (Wullner et al. 2005)
	SCA17 [^{18}F]FDG:↓metabolism in basal ganglia (Brockmann et al. 2012) [^{11}C]dMP:↓Dopamine transporter levels in caudate nucleus and putamen (Brockmann et al. 2012) [^{11}C]Raclopride:↓D2 receptor levels in caudate nucleus and putamen (Brockmann et al. 2012)
SPECT	*SCA2* [^{99}mTc]TRODAT-1 SPECT:↓striatal DAT binding (Yun et al. 2011) [^{123}I]β-CIT SPECT:↓striato-cerebellar ratio (Boesch et al. 2004) [^{123}I]IBZM SPECT:↓striato-frontal IBZM binding ratio (Boesch et al. 2004) [^{123}I]FP-CIT SPECT: ↓uptake in caudate, putamen (Varrone et al. 2004)
	SCA3 [^{99}mTc]TRODAT-1 SPECT:↓nigrostriatal ratio (Yen et al. 2000) [^{99}mTc]HMPAO SPECT:↓perfusion in cerebellar hemispheres (Etchebehere et al. 2001), inferior (Etchebehere et al. 2001) and superior (Etchebehere et al. 2001) frontal lobe (Etchebehere et al. 2001), lateral temporal lobe (Etchebehere et al. 2001), parietal lobe (Etchebehere et al. 2001), vermis (Etchebehere et al. 2001) [^{99}mTc]ECD SPECT:↓perfusion in bilateral cerebellum (Braga-Neto et al. 2016), vermis (Braga-Neto et al. 2016) [^{123}I]iomazenil SPECT:↓binding in cerebellum (Ishibashi et al. 1998), cerebral cortex (Ishibashi et al. 1998), thalamus (Ishibashi et al. 1998), striatum (Ishibashi et al. 1998)
	SCA6 [^{99}mTc]ECD SPECT:↓perfusion in cerebellar hemisphere (Honjo et al. 2004), cerebral vermis (Honjo et al. 2004)
	SCA17 [^{99}mTc]TRODAT-1 SPECT:↓striatal DAT binding (Yun et al. 2011)

Modified from Chen et al. (2021) and Brooker et al. (2021)

3.1.1 MRI

The primary neuroimaging finding in patients with SCAs is the atrophy of the cerebellum. The assessment of the reduction of volume can be done using either region of interest (ROI)-based analysis (Guerrini et al. 2004; Reetz et al. 2018; Stefanescu et al. 2015; Brockmann et al. 2012; Eichler et al. 2011; Etchebehere et al. 2001) or voxel-based morphometry (VBM) (Reetz et al. 2013; Kang et al. 2014; D'Abreu et al. 2012; Goel et al. 2011; Guimaraes et al. 2013; Reetz et al. 2010; Schulz et al. 2010). Although the cerebellum is the most commonly affected brain region in SCAs, MRI has demonstrated volume changes in the brainstem (Guerrini et al. 2004; Reetz et al. 2018; Eichler et al. 2011; Reetz et al. 2013; Kang et al. 2014; Guimaraes et al. 2013; Schulz et al. 2010), especially pons (Reetz et al. 2018; Goel et al. 2011; Schulz et al. 2010), and basal ganglia (Reetz et al. 2013).

The degree of cerebellar atrophy correlates with the severity of ataxia in SCA1 (Guerrini et al. 2004; Reetz et al. 2013; Goel et al. 2011; Schulz et al. 2010), SCA2 (Guerrini et al. 2004; Reetz et al. 2018; Goel et al. 2011), SCA3 (Stefanescu et al. 2015; Eichler et al. 2011; Etchebehere et al. 2001; Reetz et al. 2013; Kang et al. 2014; D'Abreu et al. 2012; Goel et al. 2011; Guimaraes et al. 2013; Schulz et al. 2010), SCA6 (Stefanescu et al. 2015; Eichler et al. 2011; Reetz et al. 2013; Schulz et al. 2010), and SCA17 (Brockmann et al. 2012; Reetz et al. 2010). The cross-section area of the spinal cord at C2 and C3 levels also negatively correlate with SARA severity in SCA1 (Martins Jr. et al. 2017). These findings indicate that the volume of the cerebellum and other parts of the central nervous system may be used to track disease progression in SCAs.

Can these neuroimaging findings identify structural changes prior to the symptom onset? Indeed, MRI showed volume reduction in the cerebellum and brainstem in pre-symptomatic SCA1 (Jacobi et al. 2013b) and SCA2 (Reetz et al. 2018; Jacobi et al. 2013b; Nigri et al. 2020) and the cerebellum and caudate nucleus in pre-symptomatic SCA17 (Brockmann et al. 2012). A reduction of the spinal cord area at C2 and C3 levels can also be detected in subjects with pre-symptomatic and symptomatic SCA3 (Faber et al. 2021). Notably, the degree of reduction is more severe in symptomatic SCA3 patients. Therefore, MRI can be more sensitive than clinical rating scales to measure disease progression, which has been demonstrated in SCA1, SCA2, SCA3, and SCA7 (Adanyeguh et al. 2018). The ability to detect changes in the pre-symptomatic stage allows studies to test for disease-modifying therapies before symptom onset.

In addition to grey matter visualized by volume analysis, white matter is also studied in SCAs. For example, diffusion tensor imaging showed white matter involvement finding loss of fraction anisotropy in SCA2 and SCA3 (Guimaraes et al. 2013; Mascalchi et al. 2015).

The patterns of cerebellar degeneration in particular cerebellar lobules seem to differ among SCAs (Guerrini et al. 2004; Stefanescu et al. 2015; Reetz et al. 2013; Wang et al. 2007). Therefore, a detailed analysis of each lobule may provide information regarding the different degenerative processes of each SCA, for example, lobules VIII and XI are affected more in SCA1 but not in SCA3 and SCA6 (Reetz et al. 2013).

3.1.2 MRS

MRS may detect chemical changes that precede the structural alterations seen in MRI. Commonly studied metabolites include N-acetylaspartate (NAA, a marker of neuronal density and function), creatine/phosphocreatine (Cr, a metabolism marker), choline compounds (Cho, a marker of synthesis and degradation of cell membranes), and myoinositol (a marker for gliosis). The reduction in NAA/Cr and NAA/Cho ratios thus are markers for neurodegeneration and have been shown in the cerebellum of SCA1 (Mascalchi et al. 1998; Lirng et al. 2012), SCA2 (Lirng et al. 2012; Viau et al. 2005), SCA3 (Lirng et al. 2012; Lei et al. 2011), SCA6 (Lirng et al. 2012; Hadjivassiliou et al. 2012), and SCA17 (Lirng et al. 2012) patients. MRS is an important biomarker because similar biochemical findings in the cerebellum were demonstrated in both patients and a mouse model for SCA1 (*Atxn1*$^{154Q/2Q}$). Hence, MRS findings are translatable. In particular, such changes can be tracked spanning the pre-symptomatic and symptomatic stages (Friedrich et al. 2018). Therefore, therapies that are effective in this mouse model can be studied in clinical trials in SCA1 patients and be monitored with the same MRS biomarkers.

3.1.3 Functional MRI (fMRI)

Although fMRI has not been extensively adopted in SCAs, the resting-state fMRI can assess the oxygen consumption of the cerebellum, and it was found to be reduced in the cerebellar cortex and the deep cerebellar nuclei in SCA6 (Stefanescu et al. 2015).

3.1.4 PET

PET provides important information in metabolic changes, such as glucose metabolism and integrity of the dopaminergic axis. The PET tracer, [^{18}F]fluorodeoxyglucose ([^{18}F]FDG), measures the tissue uptake of glucose, thus reflecting the overall tissue metabolism. Reduction of [^{18}F]FDG uptake of the cerebellum can be seen in SCA1 (Gilman et al. 1996; Wullner et al. 2005), SCA2 (Wang et al. 2007; Wullner et al. 2005; Oh et al. 2017), SCA3 (Wang et al. 2007; Wullner et al. 2005; Soong et al. 1997; Soong and Liu 1998), SCA6 (Wang et al. 2007; Wullner et al. 2005; Oh et al. 2017; Soong et al. 2001), and in pre-symptomatic SCA3 patients (Soong and Liu 1998). Therefore, PET may be used to monitor the progression of disease in SCA patients (Goel et al. 2011). Several PET ligands, such as [^{11}C]D-threo-methylphenidate or [^{11}C]raclopride, which interrogate the involvement of the dopaminergic axis, can be useful to study SCAs with parkinsonian symptoms, such as SCA2, SCA3, and SCA17 (Brockmann et al. 2012; Wullner et al. 2005).

3.1.5 SPECT

SPECT has been applied in ataxia studies to study the dopaminergic system and overall brain perfusion. SPECT techniques have been used to study dopamine deficiency in Parkinson's disease, but the dysfunction of dopamine reuptake can also be used to assess the dopamine uptake in SCAs, especially those that can be associated with parkinsonism, such as SCA2 (Yun et al. 2011), SCA3 (Yen et al. 2000), and SCA17 (Yun et al. 2011). [^{99}mTc]ECD SPECT (technetium-^{99}m N,N-1,2-ethylene diylbis-L-cysteine diethyl ester dihydrochloride or ethyl cysteinate dimer) showed a perfusion reduction in SCA3 (Braga-Neto et al. 2016) and SCA6 (Honjo et al. 2004).

3.2 Fluid Biomarkers

Although it is still unclear how each mutation in SCAs leads to the clinical phenotype, various biochemical alterations can be detected in blood or cerebrospinal fluid (CSF) (Table 4). These changes can serve as biomarkers for tracking disease progression or testing target engagement in clinical trials studying therapeutic interventions.

Similar to many neurodegenerative disorders, patients with SCAs also have axonal degeneration. Tau protein level, a biomarker for axonal degeneration, is reduced in the CSF of SCA2 patients (Brouillette et al. 2015). Neurofilament light chain, another marker for neurodegeneration, is increased in the serum/plasma of SCA1 (Wilke et al. 2018; Coarelli et al. 2021), SCA2 (Coarelli et al. 2021), SCA3 (Wilke et al. 2018; Coarelli et al. 2021; Li et al. 2019; Prudencio et al. 2020; Peng et al. 2020; Wilke et al. 2020), and SCA7 (Coarelli et al. 2021). A study demonstrated similar findings collectively in SCA1, 2, 3, 6, 7, and 17, compared with controls (Shin et al. 2021). The level of neurofilament light chain in CSF is also increased in SCA3 patients (Li et al. 2019; Prudencio et al. 2020). Notably, the levels of neurofilament light chain in the CSF and serum of SCA3 patients correlate with each other (Li et al. 2019), making the neurofilament light chain a peripherally accessible indicator for neurodegeneration in SCA3. In addition, the level of increment can be seen in pre-symptomatic SCA3 patients in CSF (Li et al. 2019) and plasm (Li et al. 2019; Prudencio et al. 2020). A recent study also demonstrated elevated plasma neurofilament light chain in pre-symptomatic carriers of SCA1, SCA2, SCA3, and SCA7 (Coarelli et al. 2021). Hence the change of neurofilament light chain level precedes the symptom onset. Two studies demonstrated that the serum level of neurofilament light chain is highest in symptomatic SCA3 patients, followed by pre-symptomatic SCA3 subjects, and lowest in controls (Peng et al. 2020; Wilke et al. 2020). The elevation of serum neurofilament light chain is estimated to precede the clinical symptoms by 7.5 years (Wilke et al. 2020). Similar findings were identified in a mouse model of SCA3, strengthening the idea that the levels of neurofilament reflect a degenerative process driven by mutant *ATXN3* (Wilke et al. 2020). Although the alterations of neurofilament light chain level can be found in other

Table 4 Summary of fluid biomarkers in SCA

Biomarker	Findings
Poly-Q expanded ataxin-3	↑ in PBMC of SCA3 (Wilke et al. 2020) ↑ in plasma and CSF of SCA3 (Prudencio et al. 2020) ↑ in PBMC of pre-symptomatic SCA3 (Wilke et al. 2020) ↑ in plasma and CSF of pre-symptomatic SCA3 (Prudencio et al. 2020) ↑ in CSF of symptomatic SCA3 vs. pre-symptomatic SCA3 (Prudencio et al. 2020)
Catalase activity	↑ in serum of SCA3 (Pacheco et al. 2013)
CHIP	↑ in serum of SCA3 (Hu et al. 2019) ↑ in CSF of SCA3 (Hu et al. 2019)
Oxidation of DCFH-DA	↑ in serum of symptomatic and pre-symptomatic SCA3 (de Assis et al. 2017)
Eotaxin	↑ in serum of asymptomatic SCA3 vs. pre-symptomatic SCA3/ controls (da Silva et al. 2016)
GFAP	↑ in serum of SCA3 (Shi et al. 2015)
Glutathione peroxidase activity	↓ in serum of symptomatic and pre-symptomatic SCA3 (de Assis et al. 2017)
IGFBP-1	↑ in serum of SCA3 (Saute et al. 2011)
IGFBP-3	↓ in serum of SCA3 (Saute et al. 2011)
IGF-1/IGFBP-3 molar ratio	↑ in serum of SCA3 (Saute et al. 2011)
Insulin	↓ in serum of SCA3 (Saute et al. 2011)
miRNA	↑ miR-34b (Shi et al. 2014) in serum of SCA3 ↑ miR-7014 in CSF of SCA3 (Hou et al. 2019) ↑ 71 miRs in plasma of SCA7 (Borgonio-Cuadra et al. 2019) Alterations of miRs in plasma of early onset SCA7 vs. adult onset SCA7 (Borgonio-Cuadra et al. 2019) ↓ miR-25 (Shi et al. 2014), miR-29a (Shi et al. 2014), miR-125b (Shi et al. 2014) in serum of SCA3 ↓ miR-7014 (Hou et al. 2019) in plasma of SCA3 Different expression of various exosomal miRs in plasma and CSF of SCA3 (Hou et al. 2019)
Neurofilament light chain	↑ in serum of SCA1 (Wilke et al. 2018) and SCA3 (Wilke et al. 2018, 2020; Li et al. 2019) ↑ in plasma of pre-symptomatic carriers of SCA1 (Coarelli et al. 2021), SCA2 (Coarelli et al. 2021), SCA3 (Coarelli et al. 2021; Li et al. 2019; Wilke et al. 2020), and SCA7 (Coarelli et al. 2021) ↑ in CSF of SCA3 (Li et al. 2019)
NSE	↑ in serum of SCA3 (Zhou et al. 2011; Tort et al. 2005)
Phosphorylated neurofilament heavy chain	↑ in serum of SCA3 (Wilke et al. 2020)
S100B	↑ in serum of SCA3 (Zhou et al. 2011)
Superoxide dismutase activity	↓ in serum of symptomatic and pre-symptomatic SCA3 (de Assis et al. 2017)
Tau	↑ in CSF of SCA2 (Brouillette et al. 2015)
Valine, leucine, and tyrosine	↓ in plasma of SCA7 (Nambo-Venegas et al. 2020)

Modified from Chen et al. (2021)

CHIP carboxyl terminus of the Hsp70-interacting protein, *DCFH-DA* 2′,7′-dichlorofluorescein diacetate, *GFAP* glial fibrillary acidic protein, *GSH-Px* glutathione peroxidase, *IGFBP* insulin-like growth factor-binding protein, *IGF* insulin-like growth factor, *miRNA* microRNA, *NSE* neuron-specific enolase, *PBMC* peripheral blood mononuclear cell, *SOD* superoxide dismutase

neurodegenerative disorders, SCA patients are often in their 30s to 50s and less likely to have other co-existing late-onset neurodegenerative disorders (e.g., Parkinson disease or Alzheimer disease) that may confound the interpretation.

Because the expression of abnormal poly-Q is thought to be crucial in the pathogenesis of CAG-repeat SCAs, reducing the expression of poly-Q has been the main goal for gene therapies or ASO-based therapies. To monitor the efficacy and target engagement of such interventions, it is crucial to develop an assay that can detect the level of abnormal poly-Q. An immunoassay based on time-resolved fluorescence resonance energy transfer can detect abnormal ataxin-3 with expanded poly-Q in blood-derived mononuclear cells harvested from both pre-symptomatic and symptomatic SCA3 patients (Gonsior et al. 2020). However, this assay can only detect ataxin-3 in the mononuclear cells and not ataxin-3 in the serum or CSF. On the other hand, an electrochemiluminescence immunoassay using the Meso Scale Discovery system (Gendron et al. 2017a; Gendron et al. 2017b) can identify elevated levels of abnormal ataxin-3 in the plasma and CSF in both pre-symptomatic and symptomatic SCA3 patients (Prudencio et al. 2020). Therefore, the level of ataxin-3 with abnormally expanded poly-Q can serve as a fluid biomarker to test target engagement for SCA3 in clinical trials that reduce the expression of abnormal ataxin-3.

Another potential biomarker is an endogenous binding partner of the mutant ataxin-3, the carboxyl terminus of Hsp-70 interacting protein (CHIP), a co-chaperone protein. CHIP level is elevated in both serum and CSF of SCA3 patients, indirectly reflecting mutant ataxin-3 level (Hu et al. 2019).

Inflammation can occur as the result of neurodegeneration in SCAs. The protein level of an inflammatory cytokine, eotaxin, is elevated in the serum of asymptomatic SCA3 subjects compared to controls and symptomatic SCA3 patients (da Silva et al. 2016). Therefore, eotaxin level can potentially serve as a biomarker to track the transition from the pre-symptomatic to the symptomatic stage in SCA3 patients, which eotaxin level is expected to progressively reduce as the disease progresses.

Biomarkers reflecting activation of astrocyte, gliosis, and neuronal damage have been reported in SCA3. Glial fibrillary acidic protein (GFAP), a marker for astrocytes, is elevated in the serum of SCA3 patients (Shi et al. 2014), suggesting astrogliosis occurs in the pathogenesis of SCA3. In agreement with this finding, another marker for astrocyte, S100B, is also increased in the serum of SCA3 patients (Zhou et al. 2011). Neuron-specific enolase (NSE), a marker for neuronal damage, is increased in the serum of SCA3 patients, and it may be used to track the severity of neurodegeneration in SCA3 (Zhou et al. 2011; Tort et al. 2005).

It has been reported that oxidative stress is involved in the pathogenesis of SCA3 (Araujo et al. 2011; Weber et al. 2014; Yu et al. 2009). The activity of catalase, a marker for oxidative stress, is increased in the serum of SCA3 patients (Pacheco et al. 2013). The oxidation of DCFH-DA, an artificial substrate to measure the degree of oxidation, is increased in the serum of pre-symptomatic SCA3 patients and even higher in symptomatic SCA3 patients (de Assis et al. 2017), suggesting a correlation between oxidative burden and disease severity. On the contrary, the enzymes for clearing oxidative radicals, glutathione peroxidase (de Assis et al.

2017), and superoxide dismutase (de Assis et al. 2017), are decreased in the serum of SCA3 patients. The reductions of these two enzymes are also more pronounced in symptomatic SCA3 patients than pre-symptomatic SCA3 patients (de Assis et al. 2017), further supporting the role of oxidative stress in the disease progression.

Patients with poly-Q disorders commonly present with insulin resistance, which is thought to result from reduced expression of insulin-like growth factor 1 (IGF-1) due to poly-Q peptides (Craft and Watson 2004). Thus, the levels of IGF-1 and its binding partners, IGFBP1 and IGFBP3, are potential biomarkers for SCAs. SCA3 patients have higher serum levels of IGFBP1 and IGF-1/IGFBP-3 ratio compared with healthy controls, while serum levels of IGFBP-3 and insulin levels are reduced (Saute et al. 2011).

Other biomarkers that have been studied include the levels of amino acids and microRNAs (miRNAs). Levels of valine, leucine, and tyrosine are reduced in the plasma of SCA7 patients (Nambo-Venegas et al. 2020). Altered levels of various miRNAs have been reported in serum, plasma, or CSF in SCA3 (Shi et al. 2014; Hou et al. 2019) and SCA7 (Borgonio-Cuadra et al. 2019) patients. The main limitation of the circulating miRNA studies is the small sample sizes. Further validation is necessary.

Most fluid biomarkers studies are biased toward SCA3, the most common SCA globally, with only few studies conducted in patients with SCA1, 2, and 7. Whether the findings from SCA3 can be generalized to other SCAs requires validation with future studies.

3.3 Physiology Biomarkers

Although the pathology of SCA primarily involves the cerebellum, brain areas receiving projections from the cerebellum may also be affected. Dysfunctions of the corresponding brainstem and cortical regions can be investigated with vestibulo-oculography (VOG), brainstem auditory-evoked potential (BAEP), visual-evoked potential (VEP), somatosensory-evoked potential (SSEP), and motor-evoked potential (MEP) (Table 5).

VOG can quantitatively measure oculomotor dysfunction in SCAs. For example, the speed of saccade in SCA2 patients has been shown to be around 200 °/s compared with >400 °/s in healthy controls (Buttner et al. 1998). SCA3 patients have prolonged reflex latency (Luis et al. 2016). Patients with SCA1 (Kim et al. 2013), SCA3 (Kim et al. 2013; Wu et al. 2017), and SCA6 (Kim et al. 2013) may have gaze-evoked eye nystagmus, dysmetric saccade, and square-wave jerks. These gaze-evoked eye movements occur more frequently in symptomatic SCA3 patients compared to pre-symptomatic SCA3 patients (Wu et al. 2017). Gaze-evoked nystagmus is not seen in patients with SCA2 likely because the impaired fast saccade prevents the generation of the saccadic corrective phase of nystagmus (Buttner et al. 1998).

Table 5 Physiological biomarkers of SCAs

Method	Findings
BAEP	Prolonged absolute III and V latencies and interpeak I–III latency in SCA2 (Velazquez Perez et al. 2007) Prolonged absolute I and III latency in SCA6 (Kumagai et al. 2000)
MEP	↑ CMCT in SCA1 (Jhunjhunwala et al. 2013; Yokota et al. 1998; Schwenkreis et al. 2002), SCA2 (Jhunjhunwala et al. 2013), SCA3 (Jhunjhunwala et al. 2013; Farrar et al. 2016) ↑ RMT in SCA1 (Jhunjhunwala et al. 2013; Yokota et al. 1998; Schwenkreis et al. 2002), SCA3 (Jhunjhunwala et al. 2013)
SSEP	Loss of or prolonged P40 in SCA1 (Abele et al. 1997), SCA2 (Velazquez Perez et al. 2007; Abele et al. 1997), and SCA3 (Abele et al. 1997) Prolonged P40 seen more often in SCA3 (69%) and SCA2 (23%) but not in SCA1 (Abele et al. 1997)
VEP	Prolonged P100, more commonly seen in SCA1 than SCA3 (Abele et al. 1997)
VOG	Gaze-evoked nystagmus and dysmetric saccade ↑ in SCA1 (Kim et al. 2013), SCA3 (Kim et al. 2013; Wu et al. 2017), and SCA6 (Kim et al. 2013) Gaze-evoked nystagmus ↑ in symptomatic SCA3 vs. pre-symptomatic SCA3 (Wu et al. 2017) ↓ Saccade velocity in SCA2 (Buttner et al. 1998) SWJ/SWO ↑ in SCA3 (Kim et al. 2013; Wu et al. 2017) Downward nystagmus in SCA6 (Kim et al. 2013) ↑ VOR latency in SCA3 (Luis et al. 2016)

Modified from Chen et al. (2021)
BAEP brainstem auditory evoked potential, *CMCT* central motor conduction time, *MEPs* motor evoked potential, *RMT* resting motor threshold, *SSEP* somatosensory evoked potential, *SWJ* square-wave jerk, *SWO* square-wave oscillation, *VEP* visual evoked potential, *VOG* video-oculography, *VOR* vestibulo-ocular reflex

Prolonged absolute latencies for peaks III and V in BAEP have been found in SCA2 (Velazquez Perez et al. 2007), while prolonged absolute latencies for peaks I and III have been demonstrated in SCA6 (Kumagai et al. 2000). SSEP demonstrated a disruption of the integrity of the posterior column in SCA1, 2, and 3 patients with prolonged or loss of P40 latency from tibial nerve stimulation. Prolonged P40 latency was seen more commonly in SCA3 (69%) than SCA2 (23%), while not found in SCA1 patients (Abele et al. 1997). Prolonged P100 latency in VEP can be seen in both SCA1 and SCA3 patients, while more frequently occur in SCA1 vs. SCA3 (78% vs. 25%) (Abele et al. 1997).

Prolonged central motor conduction time (CMCT) with MEP can be seen in SCA1 (Jhunjhunwala et al. 2013; Yokota et al. 1998; Schwenkreis et al. 2002), SCA2 (Jhunjhunwala et al. 2013), SCA3 (Jhunjhunwala et al. 2013; Farrar et al. 2016), and SCA6 (Lee et al. 2003), suggesting dysfunctions of the descending motor pathway. Patients with SCA1 (Jhunjhunwala et al. 2013; Yokota et al. 1998; Schwenkreis et al. 2002) and SCA3 (Jhunjhunwala et al. 2013) are found to have increased resting motor threshold (RMT), suggesting reduced corticospinal excitability.

These physiological assessments can provide objective measures to the function of the brain circuit. However, these tests have not been routinely implemented in clinical practice or clinical trials for SCAs. Most importantly, the correlation between these parameters and neurological symptoms has not been well established. Shall such correlation be validated, incorporating these physiological biomarkers into SCA clinical trial design will be very valuable.

Another potential biomarker is error-based learning mediated by the cerebellum, which has been studied by neuroscientists for decades. An example is implementing an error in the visual input to perturb hand-reaching tasks and measuring the rate of error correction by the subject (Gibo et al. 2013; Criscimagna-Hemminger et al. 2010; Butcher et al. 2017; Honda et al. 2018, 2020). Although the clinical assessment of finger-to-nose test requires the involvement of error correction, assessment for error-based learning has not been formally implemented. Additionally, efforts have been made to develop quantitative kinematic-based measurements of limb movements and gait in patients with ataxia (Honda et al. 2018; Lee et al. 2015; Bhanpuri et al. 2014; Aprigliano et al. 2019; Bakhti et al. 2018; Earhart and Bastian 2001; Hashimoto et al. 2015; Matsuda et al. 2015; Morton and Bastian 2006; Tran et al. 2019). Although the goal is to obtain an objective assessment for disease severity-related physiology measurement, standardized physiological measurements across institutions and perform data analysis will be required and valuable to provide additional information on the overall cerebellar and related brain circuitry.

4 Conclusion

This chapter summarizes the rating scales used in CAG-repeat SCAs and the recent development in biomarkers. Rating scales provide clinical assessments, while different biomarkers can deliver objective measurements to imaging, biochemistry, and physiology parameters that may help track disease severity, rate of progression, or therapeutic responses.

The natural history studies of SCAs in both the United States (Ashizawa et al. 2013) and Europe (Jacobi et al. 2011) have set the foundation for clinical trial readiness for SCAs (Lin et al. 2020), and several potential therapeutic targets have been identified. Combining the appropriate rating scale and multimodal biomarkers will ensure that clinical trials are designed rigorously with proper clinical assessment, therapeutic interventions indeed engage the expected targets, and the results will identify therapies for patients with SCAs.

Funding Information Dr. Lin has received funding from the National Ataxia Foundation. Dr. Kuo has received funding from the NIH (R01NS104423, R01NS118179, R03NS114871, and R13NS117005), Brain Research Foundation, National Ataxia Foundation, Parkinson's Foundation, and International Essential Tremor Foundation.

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Clinical Rating Scales for Ataxia

Tanja Schmitz-Hübsch

Abstract Clinical rating scales for ataxia yield a semi-quantitative measure of disease severity. Rating is based on standardized scoring, usually applied on standardized motor tests. Generic ataxia scales such as the International Cooperative Ataxia Rating Scale (ICARS) or the Scale for the Assessment and Rating of Ataxia (SARA) aim to assess ataxia independent of etiology. Disease-specific scales such as Friedreich Ataxia rating Scale (FARS) or the Unified Multiple Systems Atrophy Rating Scale (UMSARS) include a wider spectrum of specific features extending beyond ataxia. For use as an outcome in interventional trials, proof of reliability at retest is a prerequisite and prior data on the evolution of scores over time in the target group are useful for study planning. Remote application by video rating has been explored. Benchmarks of minimally important change or within-study validation against patient report are important to interpret the relevance of observed changes. Additional measures may be applied to capture treatment effects more comprehensively, for example, in the domains of executive functions, affect regulation, fatigue, or autonomic functions.

Keywords Clinical rating · SARA · FARS · ICARS · UMSARS · Responsiveness · Smallest detectable change · Minimally important difference · Timed tests · Videorating

1 Introduction

Clinical rating scales aim to describe a disease process based on severity judgements of clinical signs or symptoms by qualified raters, using standardized procedures of both, testing and rating. Thus, clinical rating is closely related to neurological exam per se, which has a prominent role in phenotyping of ataxias. Such phenotype description considers motor signs of cerebellar dysfunction in the first place, but

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_10

also needs to consider involvement of other structures of central and peripheral nervous system. Consistent with this, clinical rating scales in the field of ataxia can be classified as follows: generic ataxia rating scales that aim to assess severity of ataxia as the common pathology shared by ataxia disorders or disease-specific rating scales which aim to comprehensively assess the clinical manifestation of specific ataxia disorders, for example, Friedreich's ataxia (FRDA) or Multiple Systems Atrophy cerebellar type (MSA-C), but may not be suitable for other ataxias. Another important notion for clinical rating in ataxia is the wide age spectrum of those affected, which spans from congenital forms to geriatric populations, in which clinical rating may be confounded by motor development or normal aging and comorbidities, respectively. Thus, clinical rating scales for ataxia need to be applicable for clinically heterogeneous populations or otherwise need to specify their target group. Still, dealing with the effects of non-cerebellar signs or comorbidities on test performance remains a challenge in the application not only of clinical rating scales for ataxia but also instrumented assessment of motor functions.

Clinical rating scales can be conceived as diagnostic instruments that capture the effects of an underlying disease process at the level of clinically manifest signs and body functions, that is, the impairment level. As a framework for the specific levels addressed, terminology may refer to the international classification of functioning, disability, and health (www.who.int/standards/classifications/international-classification-of-functioning-disability-and-health), which may also serve to determine the level on which effects of novel therapeutic interventions are expected to occur (Fig. 1). For example, the progressive loss of Purkinje cells or accumulation of intracellular aggregates caused by pathogenic genetic variants may be attributed as the structural correlate of the disease process and measured as loss of cerebellar

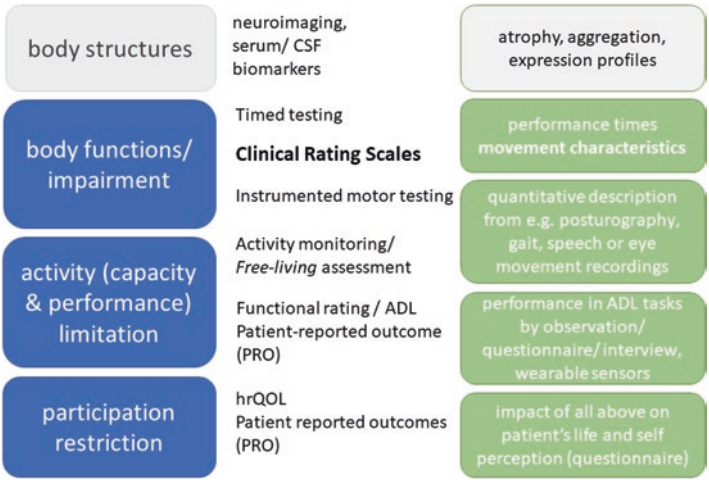

Fig. 1 Positioning of ataxia outcomes (middle column, examples or explanation in right column) in relation to the framework of the International Classification of Functioning, Disability and Health (left column)

volume captured by magnetic resonance imaging (MRI). Clinically, this pathology may result in ataxia and manifest as inability to walk in tandem or difficulty in pointing movement, captured by clinical rating. Patient experience of functional impairment, activity limitations, or restrictions in participation can be conceived as related to disease severity assessed by clinical ratings levels of assessment in fact may show overlap, for example, of walking function and perceived limitations of walking capacity. However, it is important to understand that such relations need not be strong nor linear. For example, remarkable cerebellar atrophy may be present even at the time of first and mild clinical manifestation in some hereditary ataxias and effects of intervention on aggregate formation need not be accompanied by clinical improvement. In line with this, regulatory authorities generally strengthen the use of clinically meaningful primary outcomes for pivotal efficacy trials and as a prerequisite of drug approval. Consequently, clinical rating scales have a pivotal position in this process. They may serve as a primary outcome for interventional trials, if certain criteria are met—see below–or may support the validity of effects observed on other markers, for example, MRI, motor or serum biomarkers. Recent guidance of the Food and Drug Administration for the acceptability of clinical outcome assessments (www.fda.gov/about-fda/clinical-outcome-assessment-coa-frequently-asked-questions) stresses consideration of patient experience and patient relevance of changes observed. This challenges the traditional view that amelioration or even prevention of the signs of disease would be of immediate relevance to those affected. However, the assessment of patient experience and its relation to chronic disease progression and clinical ratings in ataxia is still on debate (Riazi et al. 2006; Maas et al. 2021a; Maas and van de Warrenburg 2021)—see below—and patient-report is hitherto generally rare as a primary outcome in movement disorders.

This chapter aims to give an overview of clinical rating scales for ataxias and some guidance on the choice of clinical rating scales as trial outcomes. As a supplement to this, it also includes a summary description of complementary measures related to clinical ratings, that is, patient-reported outcomes (PRO), and motor performance measures.

2 Clinical Rating Scales for Ataxia—Remarks on Validation

The description includes both general ataxia rating scales and rating scales developed for specific phenotypes. The latter extend assessment and rating beyond the features of cerebellar ataxia to include, for example, features of spasticity and sensory impairment for FRDA (FARS) or Parkinsonian features and autonomic functions for MSA (UMSARS). The different scales are presented with respect to structure and weighting and are sequenced along date of publication. This way, readers may also trace the evolution of validation concepts over time. Results of validation are summed up for the domains of reliability, validity, and responsiveness (Mokkink et al. 2010; Hobart et al. 2007). In this context, internal responsiveness as

the ability to detect change may be demonstrated by mean score change over timeframes, in which observation of change would be expected, usually 6 months to 2 years in a neurodegenerative disease. Such changes are usually standardized to either standard deviation of baseline, yielding effect sizes (ES), or to standard deviation of change, yielding standardized response mean (SRM). This way the magnitudes of change can be compared between different types of outcomes. Both, ES and SRM, are usually interpreted according to Cohen's arbitrary criteria as 0.2 = small, 0.5 = moderate, and 0.8 = large internal responsiveness. However, interpretation of effect sizes has to consider some points. When applied in neurodegenerative diseases, effect sizes reflect a feature of the scale (responsiveness) but also a feature of the population (rate of disease progression and score variance within the sample). Further, the score changes observed should be interpreted against two important metrics: smallest detectable change (SDC) and benchmarks for minimal clinically important difference (MID). These metrics need to be defined from longitudinal observations and most often use patient global ratings of change as criterion. This way, the difference in observed score changes between those who experience worsening versus those without worsening (according to patient global ratings of change) can aid to estimate of clinically relevant change: score changes outside the 95% confidence limits of those observed in the stable group are used as one of several defintions of MID. If patient global ratings of change are dichotomized, receiver operating characteristics can be applied to determine the amount of change in clinical rating scale score that would most accurately classify the sample according to patient ratings. In contrast, the SDC is related to the standard error of measurement (SEM) and can be understood as a cutoff, above which score changes can be confidentially considered above measurement error. The SDC can be defined from score variance in re-test in subjects assumed to be stable over time (e.g., according to patient global ratings of change) in samples of appropriate size. If MID or SDC is reported for clinical rating scales, it is usually provided for the total score only and responsiveness may differ at item level (O'Connor et al. 2004). SDC for intra-individual observation is higher, while SDC for group observations can be derived as individual SDC divided by $\sqrt{n}$ of sample. If SDC were determined appropriately, score changes below SDC should strictly not be considered meaningful. With respect to MID, however, several reviews pointed out that MID should not be applied too rigidly, as it depends on the type of anchor used, may depend on context of use, baseline value, and also on direction of change (O'Connor et al. 2004; de Vet et al. 2006; Norman et al. 2003; Revicki et al. 2008). When selecting a clinical rating scale for a given sample, one should carefully check the appropriateness of a given scale beyond the quality criteria mentioned above: has it been applied in the specific disease before? Are there major differences in patient characteristics between validation samples and intended use? Have floor or ceiling effects been observed in relevant proportions? Does the content of the scale cover the symptoms or functions of interest? A recent review on clinical rating scales for ataxia (Perez-Lloret et al. 2021) comprehensively listed the quality criteria along with past and current use in validation studies and clinical trials. Indeed, comparability with results from previous or competing trials may be a consideration but should not

neglect evidence on quality and specific acceptability of an outcome. At last, status of licensing should be checked before using an instrument in clinical trials as well as time needed to perform the test and appropriate rater training. While there is consensus that patient reported outcomes need application in valid translations, there is less consensus inhowfar translations of clinical rating scales would be useful or how this might affect their metric properties. If translated versions are used, these should undergo at least procedures for linguistic validation.

3 ICARS—The International Cooperative Ataxia Rating Scale

The scale was devised by an expert ad hoc committee in 1997 as a first international standard for the clinical rating of ataxia (Trouillas et al. 1997). Authors acknowledged that cerebellar signs may occur within a more complex syndrome in ataxia disorders, but selected items according to their assumed specificity for ataxia. The 19 items cover four domains of body functioning which were proposed as subscales of the total score: posture/gait (seven items, maximum 34 points), kinetic functions, rated on both sides (seven items, maximum 52 points), speech function (two items, maximum eight points), and oculomotor function (three items, maximum six points). The total score is built by addition of subscores and describes severity of ataxia (0 = no ataxia; 100 = most severe ataxia).

Although the original publication did not contain data on validation, the scale gained wide use for the clinical description of ataxia populations and several studies evaluated its metric properties. Results generally support reliability between raters or at retest for the total score in different ataxias, but also revealed limitations and the need for rater training. Scoring instructions were noted as imprecise for some kinetic items and interdependent ratings were noted for posture/gait items (Schmitz-Hubsch et al. 2006), Parkinsonian features seemed to contaminate ataxia rating in MSA (Tison et al. 2002), and re-test reliability was lower for speech and oculomotor items. Sufficient internal consistency was reported from samples of MSA, spinocerebellar ataxias(SCA), FRDA (Bürk et al. 2009) Multiple Sclerosis, and focal cerebellar lesions (Schoch et al. 2007). Factorial analysis did not fully support the four domains of the scale in most studies, such that use of subscales was not endorsed except for MSA and focal cerebellar lesions. Validity was shown against different clinical rating scales, disease stages or measures of activity limitations in FRDA, SCA, MSA, multiple sclerosis with ataxic symptoms (Salci et al. 2017), ataxia teleangiectasia (Nissenkorn et al. 2016), and FXTAS. The longitudinal assessment of disease progression in FRDA reported a 5-point change at 12 months, with corresponding ES of 0.26 and SRM of 0.74 (Fahey et al. 2007). Different progression rates for different disability stages and a plateau in ICARS ratings of later stage FRDA may point to non-linear properties of the scale but may also be interpreted as inherent characteristics of the disease (Tai et al. 2015a; Ribai et al. 2007).

Further exploration of linearity, that is, discriminant abilities over the whole range of scoring, has not been reported. In common with most clinical rating scales, benchmarks of smallest detectable difference or indicators of patient relevance of score changes have not been established for ICARS.

4 UMSARS—The Unified Multiple Systems Atrophy Rating Scale

This scale was developed and published in 2004 by the Multiple System Atrophy Study Group (Wenning et al. 2004). The development was triggered by the prospect of clinical trials for neuroprotective interventions and the need for a valid and reliable outcome to prove treatment efficacy in MSA. This neurodegenerative disorder clinically combines features of ataxia, Parkinsonism, pyramidal signs, and autonomic failure which are all considered in a comprehensive rating. Similar to the Unified Parkinson's Disease Rating Scale, this rating instrument was devised as a multi-dimensional scale and is structured in four parts. Part I (12 items, maximum 48 points) is rated by interview to assess impairment of bodily functions or limitations in daily activities over the past 2 weeks, regardless of the nature of the signs. Part II rates the results of a motor examination (14 items, maximum 56 points) with specific attention to features of ataxia and Parkinsonism. Of note, limb items are tested on both sides but only worse side is included in rating. Part III reports results of a standard bedside tests of orthostatic dysregulation without rating, while part IV consists of a 5-step disability rating (see below). The original publication reported high reliability for parts I and II according to Cronbach's alpha, but item-total correlations suggested some inconsistency with total scores for part I items 8 (falling) and 9 (orthostatic symptoms) and part II item 3 (oculomotor dysfunction). Inter-rater agreement was high to excellent (ICC >0.85) for the subscores, but lower at item-level for ratings of oculomotor dysfunction, muscle tone, rapid alternating movements, and finger tapping. Validity of both, part I and part II, was supported by comparison against a 3-step global disability rating. Test-retest reliability was determined as high in independent samples (Krismer et al. 2012). Subsequent use of the scale in longitudinal observational studies in a European MSA cohort reported part I/part II score changes of 6.7/9.6 at 12 months (Geser et al. 2006). Faster progression according to UMSARS in those with milder disability and shorter disease duration may be considered a feature of the disease but may also point to non-linearity of the rating. Longitudinal observation in a US sample reported lower progression rates of 3.1/4.5 in part I/part II after 12 months (May et al. 2007). The scale has also been applied in spinocerebellar ataxia (SCA) type 3 (D'Abreu et al. 2007) in which a 2.7 point worsening (part II) was observed over 13 months. In both, MSA and SCA3, correlations between UMSARS part II and ICARS ratings were high and support shared constructs.

While benchmarks for reliable change (SDC) have not been reported for this scale, one study defined MID for symptom worsening in MSA of Parkinsonian type

using receiver operating characteristics with clinical global impression of change as an anchor (Krismer et al. 2016). However, the adequacy of reported MID as 1.5/1.5 points for part I and part II, respectively, in MSA of cerebellar type remains to be shown.

5 FARS—The Friedreich Ataxia Rating Scale

This scale was developed by the North American Cooperative Ataxia Group and published in 2005 (Subramony et al. 2005). Developers of the scale aimed for a valid and potentially responsive clinical assessment in Friedreich's ataxia (FRDA). As a starting point, they acknowledge the symptom spectrum specific to this disorder and the intraindividual evolution of symptoms over time, such that clinical findings seen in earlier stages (e.g., nystagmus) may become absent at later stages despite progression of disease. They chose to assign highest weight to the assessment of gait and stance. The scale is multi-dimensional and comprises four parts. Part I consists of a 6-step functional staging of ataxia (see below). Part II targets restrictions in ADL performance in 9 items (maximum score 36), while part III provides clinical rating from a standardized motor examination (22 items, maximum score 117, limb items rated each on both sides). Scale structure suggests five subscores within part III: bulbar (maximum score 11), upper extremity (36), lower extremity (16), peripheral (26), and upright stability (28/36 in 2006 FARSn revision (Lynch et al. 2006)). Part IV comprises quantitative stopwatch tests of speech, hand function, and gait capacity. The original publication reported excellent inter-rater reliability for most scores except for part III bulbar and peripheral nerve. Correlations among the scale's parts were substantial, even in the small cohort of 14 patients, with the exception of part III bulbar. In some studies, FARS is reported as sum of parts I to III (maximum score 159). Factor analysis from independent samples did not fully support the scale structure and part III subscores (Rummey et al. 2019).

A modified version of the FARS part III—mFARS—has been proposed which excluded two bulbar items (facial and tongue atrophy) and the peripheral items (maximum score 93). This mFARS has seen increasing and preferential use over FARS part III in observational and interventional trials (Reetz et al. 2021; Xiong et al. 2020; Lynch et al. 2021). A recent cross-sectional validation of both, original and modified mFARS in a large multi-national cohort, confirmed improved construct validity (Rummey et al. 2019). Of note, modification removes the non-cerebellar items of the original scale (Fig. 2).

Reports on clinical validation in samples of more than 50 patients established correlations >0.9 of FARS part III with ICARS (Fahey et al. 2007), SARA (Bürk et al. 2009), and measures of functional disability and activities of daily living (Lynch et al. 2006). When analyzed by subscores of FARS part III, such correlations remained high only for the upright stability subscore. Data on responsiveness from longitudinal observation in larger samples (Regner et al. 2012; Friedman et al. 2010) showed convergent decline in parts I to III of the FARS. For part III, this

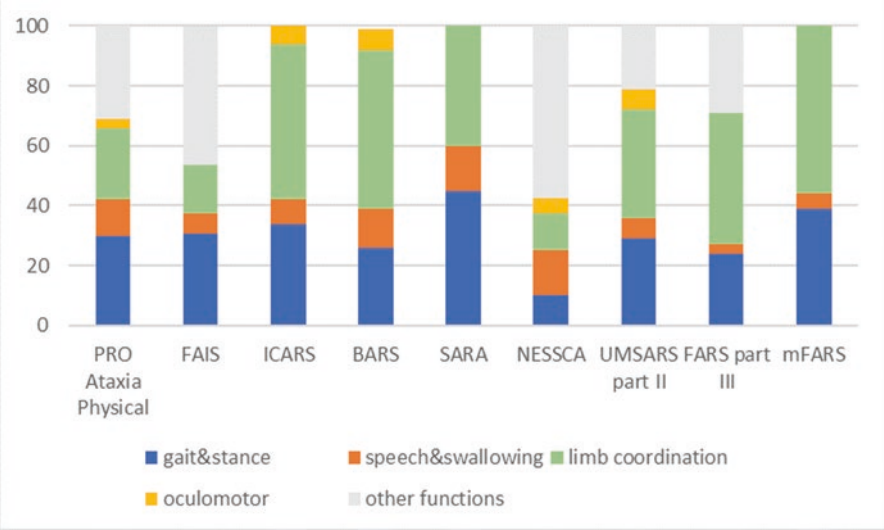

Fig. 2 Illustration of the proportional representations as percentage contribution to total score per different domains affected by ataxias for the rating scales reported herein: two patient-reported outcomes (PRO Ataxia and FAIS, left) and clinical ataxia rating scales. Other functions refer to non-ataxia signs or functions or functions not unequivocally attributable

change of 3.6/6.2 points after 1/2 years corresponds to standardized response mean (SRM) of 0.53 at 12 months and 0.84 after 2 years. This effect seemed mainly driven by limb coordination and upright stability subscores. Importantly, this study suggested ceiling effects of the scale in patients with higher disability. Moderate correlations were established between FARS part II/III and the physical component summary of the Short-Form Health Survey 36 (SF36) (Tai et al. 2017a).

Benchmarks of SDC or MID have not been formally established for FARS or mFARS, but valuable evidence can be drawn from recent RCTs which described mFARS reductions of up to −2.3 in placebo groups after 3 months observation that seems to vane thereafter (Lynch et al. 2019a, b). Based on this, a recent study used mFARS score change of ≤−1.9 as criterion for clinical improvement and reported mean mFARS reduction of −1.55 with omaveloxolone compared to mean increase of 0.85 in placebo at 48 weeks (Lynch et al. 2021).

6 SARA—Scale for the Assessment and Rating of Ataxia

Published in 2006, this scale was devised by an European consortium to address the need for a validated assessment to evaluate therapeutic interventions in spinocerebellar ataxias (Schmitz-Hübsch et al. 2006). Existing scales were not considered suitable for this purpose due to concerns on practicability and construct validity (ICARS) or disease-specific design (FARS). Therefore, SARA was designed as a

generic measure to assess cerebellar ataxia on an impairment level, that is, the level of physical functions. Item selection and rating instructions were by expert consensus and aimed for specificity for ataxia, standardized instructions, and coverage of the full range of symptom severity over the disease course. Further, the contributions of ratings in gait/stance, speech, and limb items to total score were chosen to reflect their impact on patient functioning. Of note, limb items are rated separately for each side, but only the means of both sides are included in total score. The assessment of oculomotor functions was not included in the final version of the scale, because results of a first validation trial were not supportive.

The original report contained results of scale validation in 119 subjects with spinocerebellar ataxias and 110 controls. No floor or ceiling effects were observed and all patients scored ≥1.5 on SARA total. The observation of positive ratings in 21% of controls was mainly in limb kinetic functions of the non-dominant side. Factor analysis supported the unidimensional structure of the scale. Reliability was high for the total score both between raters and at retest. At item-level, inter-rater reliability was lower (but ICC still >0.7) for items 6 (finger-nose test) and item 8 (heel-shin test) on the left side. Regression analysis were performed against global clinical ratings of ataxia severity performed on video recordings in a subset. Results supported linearity of ratings over the whole range of the scale and also linearity of score differences. Validity was shown by high convergence to ataxia disease stages (see below) as well as measures of activity limitations.

The scale has gained wide acceptance in clinical use due its practicality. Subsequent evaluations of its metric properties generally confirmed high reliability, internal consistency, and convergent validity in a heterogeneous ataxia population (Weyer et al. 2007). In patients with Friedreich's ataxia, high correlations of $r > 0.9$ were seen with ratings on ICARS and FARS (Bürk et al. 2009). Data are also available from application in different SCA, FRDA (Marelli et al. 2012), and rarer recessive ataxias (Nissenkorn et al. 2016; Traschütz et al. 2020, 2021; Bourcier et al. 2020), Niemann-Pick type C (Patterson et al. 2021), multiple sclerosis with ataxic symptoms (Salci et al. 2017), cerebellar stroke (Choi et al. 2018; Kim et al. 2011), and pediatric brain tumors (Hartley et al. 2015). Importantly, use in early onset ataxia explored the utility in children (Lawerman et al. 2016). While inter-rater reliability was high, limitations in validity were noted due to coincident non-cerebellar manifestation and motor development (Brandsma et al. 2017; Lawerman et al. 2017).

Data on scale's responsiveness are available from several long-term observational studies in SCA1, 2, 3, and 6 (Schmitz-Hübsch et al. 2010a; Jacobi et al. 2011, 2015; Ashizawa et al. 2013). For the mixed sample, SARA changes at 12 months were reported per group of patients with worsening according to patient global impression as increase of 1.69 ± 2.9 points (95% limits of confidence 1.2–2.2), which corresponds to a SRM of 0.59, while in patients who did not report worsening, SARA change was as minimal as 0.43 ± 2.1-point increase (95% confidence interval [CI] −0.2–1.1, SRM: 0.21). Authors suggested the upper limits of change in subjects without worsening, that is, a 1.2 increase in SARA, as a benchmark for minimal clinically important change and SARA change at 12 months classified those with subjective worsening with sufficient accuracy (area under the curve

[AUC]: 0.613) (Schmitz-Hübsch et al. 2010a). Natural history studies of SARA have been summarized in a recent meta-analysis comprising >1200 patients and reported striking similarity of findings from European, Asian, and US cohorts. Annual pooled SARA score increase was highest in SCA1 with 1.83 (1.46–2.20), intermediate in SCA2 with 1.40 (1.19–1.61) and SCA3 with 1.41 (0.97–1.84), and lowest in SCA6 with 0.81 (0.66–0.97). For patients with SCA3, disease progression was faster in studies located in Asia and Europe than in the US. Progression rates have also been reported from longitudinal analysis in SCA7 (Contreras et al. 2021; Marianelli et al. 2021), CANVAS (Traschütz et al. 2021), COQ8A ataxia (Traschütz et al. 2020), ARSACS (Bourcier et al. 2020), and FRDA (Reetz et al. 2016, 2021; Marelli et al. 2012). Of note, the study in ARSACS included a Delphi survey to confirm content validity. Smallest detectable change was determined as 3.5-point change on individual SARA scores by distribution-based methods in a mixed SCA sample (Schmitz-Hübsch et al. 2010a) and replication in an ARSACS sample yielded similar results (SDC 3.06). A recent study reported responsiveness to clinical improvement also in autoimmune ataxia (Damato et al. 2021). Again, useful information may be drawn from recent or future use in interventional trials. However, for the compounds studied with SARA as the clinical outcome, mean SARA score changes did not exceed SDC in neither treatment nor placebo groups, and no symptomatic effect could be established to date (Feil et al. 2021; Romano et al. 2015; Coarelli et al. 2022).

7 NESSCA—Neurological Examination Score for Spinocerebellar Ataxia

This instrument has been used by developers since 2001 and metric properties were published in 2008 (Kieling et al. 2008). The scale is designed to comprehensively capture signs of spinocerebellar ataxias, focused on SCA3, which comprises pathology of cerebellum and additional involvement of other structures. Thirteen out of 18 items cover clinical signs of ataxia (gait, limb speech, oculomotor dysfunctions) as well as pyramidal and extrapyramidal affection, lower motoneuron, and peripheral nerves. The remaining five items cover patient report on dysphagia, vertigo, sphincter function, and cramps. Total score ranges from 0 (no affection) to 40 (maximum severity). Factorial analysis supported a multi-dimensional structure of the scale. Validation data are available from the SCA3 and SCA2 cohorts, which reported sufficient internal consistency, high inter-rater reliability, while re-test reliability was not investigated. Convergent validity was demonstrated in SCA3 by correlation with disease stages (rho > 0.75). Associations with SARA ratings were high in a subgroup of the cohort ($r > 0.85$) which also applied for NESSCA subscores of ataxia ($r = 0.84$) and non-ataxia ($r = 0.76$) items. In a separate study, correlations with SARA were lower in SCA2 ($r > 0.6$) (Monte et al. 2017, 2018). Responsiveness of 1.26 point change at 12 months has been reported from a large observational

study in SCA3 (Jardim et al. 2010). However, test-retest reliability and thus standard error of measurement as well a minimal clinically important change have not been determined.

8 BARS—The Brief Ataxia Rating Scale

This scale—published in 2009—was developed as a derivative of a modified version of ICARS with the intention to increase practicality for clinical use (Schmahmann et al. 2009). Item selection was based on the evaluation of item-total correlations for the modified ICARS, but also integrated expert decision to incorporate at least one item per domains of gait, kinetic functions arm, kinetic functions leg and, speech, and eye movements. The scale consists of only five items yielding a maximum score of 30 which by design is highly correlated with ICARS and modified ICARS. According to the original publication, application in a separate cohort demonstrated high reliability (internal consistency and inter-rater reliability) and criterion validity against ICARS. Further use specifically supported practicality in children, in which high inter-rater and retest reliability were confirmed (Brandsma et al. 2014). Convergent validity was established in patients with ataxia teleangiextasia (Nissenkorn et al. 2016) and children with posterior fossa brain tumors (Hartley et al. 2015). However, no report is available from longitudinal observation and benchmarks for reliable and important change remain to be determined.

9 CCAS Scale—Cerebellar Cognitive Affective Syndrome Scale

This scale's content covers a different construct: whereas the clinical rating scales based on neurological examination target different motor functions, the CCAS targets cognitive-affective sequalae of cerebellar dysfunction that may add to the limitations and restrictions perceived by patients with ataxia. The scale was published in 2018 and developed as an office and bedside screening test (Hoche et al. 2018). The development was guided by previous description of the CCAS (Schmahmann and Sherman 1998) characterized by deficits in executive function, linguistic processing, spatial cognition, and affect regulation. The CCAS Scale comprises short standardized tests of nine cognitive functions and one composite item on affective disturbance based on clinical judgement. Cognitive tests cover semantic and phonemic fluency, category switching, verbal learning, digit span forward and backward, cube drawing, similarities, and go no-go task. Scoring instructions yield raw scores per item to form a sum score (0 = fail, 120 = highest performance). For use as a screening instrument, however, authors propose fail/pass criteria based on normative data for each item and define possible, probable, and definite CCAS if subjects

fail in one, two, or three items, respectively. Validity has been established as sensitivity and selectivity in an ataxia group of mixed etiology and pathology in comparison to controls. The original publication provided three alternative versions for repeated testing but did not provide guidance on acceptable intervals for retest. The scale has since been used in SCA2 (Rodríguez-Labrada et al. 2021), SCA3 (Maas et al. 2021b), ataxia teleangiectasia (Hoche et al. 2019), and cerebellar stroke (Chirino-Pérez et al. 2021) and supported prevalence of CCAS, confirmed high sensitivity of CCAS scale but revealed also limited specificity for single items and an influence of ataxia severity, age, and education on scale performance. The need for transcultural adaptation is under investigation (Thieme et al. 2020). Reliability and responsiveness of the scale remains to be shown in the target group of adult persons with ataxia as well as applicability and validity in children.

10 INAS—Inventory of Non-Ataxia Symptoms

This inventory was originally devised for comprehensive phenotyping and descriptive analysis in a mixed SCA cohort, in which the occurrence of non-cerebellar features had to be expected (Schmitz-Hübsch et al. 2008a). The feature content was selected by expert consensus and respective features listed for documentation by the rater as none, mild, moderate, or severe. Of note, no further instructions on testing or 4-step item-level ratings are provided. In later analyses, items were grouped according to 16 systems and presence of a sign of any severity within a group led to assignment of 1 for that group. The resulting INAS count represents the sum of affected systems and provides a rough measure of the extent of non-cerebellar affection in a single patient or study population (Jacobi et al. 2013a). An 0.37 increase of INAS count was observed at 12 months in a mixed sample of SCA1, 2, 3, and 6, but the standardized response mean was much lower (0.26) than for SARA score changes.

11 Disability Staging

Disability staging refers to a more condensed rating of overall performance or restrictions. It is not based on standard testing but rather a clinical judgement of severity with some anchor provided in scale description. Stagings are not designed to capture changes in disease status in the shorter term, but have a role as an external validation criterion in the development of clinical rating scales or may be used as a classification to stratify patient samples. The widely used 4-step ataxia disease stages were first applied in a large retrospective analysis of the natural history of ataxias (Klockgether et al. 1998). A different staging with range from 0 (no cerebellar sign) to 7 (bedridden) has been proposed as spinocerebellar degeneration functional score (SDFS) and was applied in recessive ataxias and MSA (Anheim et al. 2010;

Wirth et al. 2022). Different stagings also form part of the FARS (part I, 7-step rating) and UMSARS (part IV, 5-step rating). While ataxia disease stages and FARS staging only rely on limitations of mobility, the UMSARS part IV is more comprehensive to include any limitation.

11.1 Mobility Stages (Klockgether et al. 1998)

Stage 0	No gait difficulties.
Stage 1	Disease onset, as defined by onset of gait difficulties.
Stage 2	Loss of independent gait, as defined by permanent use of a walking aid or reliance on a supporting arm.
Stage 3	Confinement to wheelchair, as defined by permanent use of a wheelchair.

11.2 UMSARS Part IV: Global Disability Scale (Wenning et al. 2004)

Score 1	Completely independent. Able to do all chores with minimal difficulty or impairment. Essentially normal. Unaware of any difficulty.
Score 2	Not completely independent. Needs help with some chores.
Score 3	More dependent. Help with half of chores. Spends a large part of the day with chores.
Score 4	Very dependent. Now and then does a few chores alone or begins alone. Much help needed.
Score 5	Totally dependent and helpless. Bedridden.

11.3 FARS—Functional Staging of Ataxia (Subramony et al. 2005)

Increment by 0.5 may be used if the status is about the middle between two stages.

Stage 0	Normal.
Stage 1.0	Minimal signs detected by physician during screening. Can run or jump without loss of balance. No disability.
Stage 2.0	Symptoms present, recognized by patient, but still mild. Cannot run or jump without losing balance. The patient is physically capable of leading an independent life, but daily activities may be somewhat restricted. Minimal disability.
Stage 3.0	Symptoms are overt and significant. Requires regular or periodic holding onto wall/furniture or use of a cane for stability and walking. Mild disability. (Note: many patients postpone obtaining a cane by avoiding open spaces and walking with the aid of walls/people etc. These patients are grades as stage 3.0)

Stage 4.0	Walking requires a walker, Canadian crutches or two canes. Or other aids such as walking dogs. Can perform several activities of daily living. Moderate disability.
Stage 5.0	Confined but can navigate a wheelchair. Can perform some activities of daily living that do not require standing or walking. Severe disability.
Stage 6.0	Confined to wheelchair or bed with total dependency for all activities of daily living. Total disability.

12 Remote Assessment of Ataxia

Technical developments and promotion of telehealth as well as clinical need have fostered the exploration of remote assessment of ataxia. In the most simple form, patients are instructed by an application on their mobile device to record videos during performance of a set of standard motor tasks. These videos can then be transferred and rated offline by a clinical rater. This approach has been published for a five-item adaptation of SARA, the SARAhome, which includes gait, stance, speech, nose-finger test, and fast alternating hand movements (range: 0–28) (Grobe-Einsler et al. 2021). The first application demonstrated near-perfect correlations of SARAhome ratings to full SARA scores obtained at in-patient visits. The study yielded important information on the applicability of multi-point testing in the home setting and revealed considerable intra-individual variability. Variability of SARAhome recordings performed remotely once per day for a period of 14 days amounted to SARAhome differences between 1 and 5.5/28 points. This finding may be generalizable to other similar approaches of remote assessment and needs consideration when integrating remote functional assessments in trial design.

A recent pilot study in FRDA patients demonstrated the feasibility to perform video recording of modified FARS and full SARA at home with assistance of a caregiver. Reliability at repeated testing was high for SARA, FARS III, and its subscores upright stability and lower limbs (Tai et al. 2021).

As an extension of conventional videorating, consumer grade infrared cameras have been explored for their utility to remotely assess ataxic children. Recordings used the conventional hardware setup of the Microsoft Kinect V2.0 RGB-depth camera and a customized user interface and analysis software. The pilot trial reported applicability and acceptance as promising without further detail on rating (Summa et al. 2020a). The test protocol was inspired by SARA items and therefore named SaraHome (Summa et al. 2020b). Of note, SaraHome denotes a testing protocol distinct from SARAhome described above, despite similar names.

From the perspective of outcome metrics, the use of remote assessment in clinical trials will need to assure smooth patient experience and safety, counteract attrition in use, ensure sufficient data quality, that is, establish the accuracy and reliability in unsupervised application.

13 Functional Composite Scores

Scores from clinical rating scales are inherently semi-quantitative and at best interval ratings. Therefore, simple or more elaborate performance tests have been introduced as quantitative performance measures. This was expected to yield higher sensitivity to change and improve reproducibility by elimination of rater interpretation.

Different combinations of simple stopwatch tests have been explored in ataxias, based on the observation that motor incoordination results in slowing of motor performance and drawing from experience with the Functional Composite in Multiple Sclerosis. As a general drawback, achieving scores from raw data (i.e., transformation of performance times into Z-scores or other Indices) is not straightforward (e.g., decision on choice of reference population to calculate Z scores) and interpretation of change is often unresolved. Further, inability to perform a test at follow-up needs to be coded as informative missing and use is generally limited by patient's capacity to perform individual tests. Normative data should be available to estimate effects of age for interpretation. Multiple composite scores of such timed tests has been devised and explored for ataxias. Of note, results from the composite functional score described below are often reported per test component and components have also been singled out as an outcome, such as 8 m-walk (8 MW) or timed 25-ft walking test (T25FW) for walking function, 9-hole peg test (9HPT) or click test for hand function, and syllable repetition (PATA) for speech function.

The composite FARS part IV consists of three performance tests: PATA rate (speech function), time to perform the 9-hole peg test (hand dexterity function), and timed 25-foot walk test (gait function) (Subramony et al. 2005). A similar composite, the AFCS (Ataxia Functional Composite Scale), also includes low contrast visual acuity test (visual function) (Assadi et al. 2008; Lynch et al. 2005). It has been applied in SCA and FRDA with high retest reliability and strong correlation to clinical severity ratings (Lynch et al. 2006). Responsiveness in FRDA patients was reported for timeframes up to 3 years and seemed highest for the 9HPT component (Friedman et al. 2010; Tai et al. 2017b).

The CCFS (Composite Cerebellar Functional Severity Score) consists of two tests of hand coordination: 9-hole peg test and click test (du Montcel et al. 2008). Performance times are transformed into age-corrected scores. Validation reported high retest reliability and convergent validity with SARA scores in SCA patients (du Montcel et al. 2008) and FRDA (Tanguy Melac et al. 2018). Responsiveness was reported as a score change at 12 months with SRM >0.6 in SCA1, 2 and 3 (Chan et al. 2011) but no minimally important change was reported. Of note, CCFS differed between mutation carriers and non-carriers in the pre-manifest phase for SCA1, 2, and 3 (Jacobi et al. 2013b).

The SCAFI (SCA Functional index) was devised for use in SCA (Schmitz-Hübsch et al. 2008b). It consists of similar tests as FARS part IV: PATA rate, 9 hole peg test, and 8 m walk at maximum speed. It uses standardized computation of Z-scores per test that also integrates codings for inability to perform.

Test-retest reliability was good in SCA and convergent validity to clinical ataxia ratings was shown in SCA1, 2, 3, and 6 (Schmitz-Hübsch et al. 2010a) and FRDA (Reetz et al. 2015). Application in at-risk cohorts suggested sensitivity in the pre-symptomatic phase of SCA2 (Jacobi et al. 2013b). Responsiveness of SCAFI was shown for a mixed cohort of SCA1, 2, 3, and 6 at 12 months, but not for its component 8 m walk or PATA. Most favorable results were seen for 9-hole peg test with SRM of 0.67, that is, superior to change in SARA scores (SRM of 0.5 in the same sample). However, 9HPT changes did not discriminate between groups with and without perceived worsening according to patient global ratings of change (Schmitz-Hübsch et al. 2010a). This may be interpreted to reflect higher sensitivity of this test to detect "sub-clinical" change, but as a consequence leaves importance or relevance of change to patients an open issue. In clinical trials, this should be accounted for by multi-dimensional assessment with complementary assessment at the level of patient perception.

As a generic measure, the NIH Toolbox for the Assessment of Neurological and Behavioral Function was proposed as a flexible assessment battery for use in neurological disorders (Gershon et al. 2010a). It contains a set of tests for dexterity, strength, balance, locomotion, and endurance that have undergone thorough validation and are provided along with recently assembled normative datasets over an age spectrum of 3–82 years. To date, no published study in ataxias referenced the NIH toolbox.

14 Instrumented Motor Testing

Impaired motor performance is well amenable not only to observer ratings but also to technical recording, which, if applied along with a standard test instruction, may be referred to as instrumented motor testing. Such recordings may yield quantitative descriptors of movement for clinical use, recently referred to as "motor biomarkers." A multitude of technical advancements (e.g., force plates, wearable inertial sensors, marker-based optical systems, and marker-free visual perceptive computing) have been used for this purpose and most often apply kinematic analyses. Though evidence is still scattered by virtue of different technologies, algorithms, and standardization of tasks, some convergent findings can be subsumed. Beyond slowing of movement—that may be detected by timed tests described above—further clinical features of ataxia may be quantified such as broadened step width while walking or increased trunk movements while standing or walking. In a review of instrumented motor testing of limb coordination, authors proposed a set of affected domains of motor performance which may help to delineate the specific pathology across different studies, devices, and metrics (Power et al. 2021). Increased variability of movement has been described in spatial and temporal domains, most often reported for locomotor stepping during walking tasks, but also for speech function (Ilg et al. 2012; Kroneberg et al. 2018; Shah et al. 2021; Schniepp et al. 2014;

Rochester et al. 2014; Vogel et al. 2020; Hickey et al. 2016; Schmitz-Hubsch et al. 2016). Intriguingly, evidence converges that subtle changes in motor biomarkers such as variability can be detected in pre-ataxic carriers, that is, persons without symptoms or clinical ratings of ataxia (Rochester et al. 2014; Vogel et al. 2020; Thierfelder et al. 2022; Ilg et al. 2016; Velázquez-Pérez et al. 2021).

Another advantage of such technology is its potential for rater-independent remote recording (see above). Unobtrusive instrumentation may even extend beyond task-based assessments to the recording of real-life activities. While commercial activity monitors are usually confined to types and amount of physical activity or step count per day (Schniepp et al. 2022), recent research developed promising approaches for quantitative gait analysis from real-life walking (Thierfelder et al. 2022; Ilg et al. 2020; Shema-Shiratzky et al. 2020). Compared to the wealth of literature in this field, only few studies systematically explored the metric properties, validity, and responsiveness of such measures (Milne et al. 2018, 2021) and they are not (yet) part of the protocols of the large natural history studies in ataxias.

15 Patient-Reported Outcomes for Ataxias

Patient-reported outcomes (PRO) refer to the assessment of patient experience, most often by self-report questionnaires, but may also be obtained by structured interview or even from caregivers. By content, PRO cover those features of disease that are amenable to patient perception, such as disturbance of motor coordination, weakness, or numbness. Importantly, for some of the features, patients only can validly report on presence or absence or severity of a specific symptom. This typically applies to the presence or the severity of pain, mood, fatigue, impairment in executive functions or behavioral change. Further, patient report is important to capture aspects of disease that may not become evident at the clinical visit. This applies to episodic phenomena such as seizures, falls, or infrequent disturbance (myoclonus, spasms) but also to disturbed sleep or other autonomous dysfunctions. Not least, patient report is essential to evaluate the impact of disease on everyday functioning and general well-being, which are conceived as the target of all medical procedures, according to the current positive definition of health endorsed by the World Health Organization (WHO). In this sense, for chronic diseases, patient-report of functioning, well-being, and life satisfaction is a valid anchor to establish the relevance of change in other outcome assessments or biomarkers or gauge the effectiveness of medical interventions. Consequently, regulatory bodies have emphasized that patient view should be considered in study planning and generally recommend inclusion of PRO as one of the study outcomes.

The term PRO subsumes a variety of instruments that can largely differ in construct and dimensionality. As a guidance, one first important distinction is whether patient report is used to identify and scale specific symptoms or combinations thereof, such as pain or depression questionnaires. Most of these instruments are specific to the symptom but not confined to use in specific diseases. Still, their

content should be checked for applicability in the population under study with respect to their disability levels.

Other PRO address the impact of disease and treatments on patient performance in activities of daily living (ADL), perceived (health-related) quality of life (hrQOL), and general well-being. All three constructs are generally conceived as somehow related to symptom severity—whether assessed with PRO or clinical rating scales—but as different in several aspects. First, they are notably subject to many other factors than disease such as role perceptions, coping, treatment settings, social and family support, lifestyle, and occupational status. Specifically for the construct of hrQOL, the WHO definition explicitly states the specific cultural context as the reference for self-perception and well-being. This makes such instruments prone to some cultural or lifestyle bias that need consideration when used in contexts different from those in which the PRO was developed. Second, PRO of ADL or hrQOL usually have a multi-dimensional structure designed to assess all but only relevant domains of the disease and users should check the applicability of the content in their target group. For example, generic hrQOL instruments usually consider the domains of physical functioning, mood, ADL, and social roles as known health-related determinants of subjective well-being and life satisfaction. For disease-specific PRO of ADL or hrQOL, the development usually integrates both knowledge on domains affected by the specific disease and knowledge of their relevance to those affected. It is inevitable, that questions on hrQOL relate to the severity of symptoms to some extent, asking for example "how much did your problem in motor coordination prevent you from …?". All this implies that the ADL type of PRO should be considered as more specific to a disease compared to generic hrQOL PROs and explains usually moderate relations to disease severity at the level of symptoms or structural level. Using a generic PRO for ADL or hrQOL can be a good choice to enable comparison between ataxias or across medical conditions or with population level data. However, PRO developed for use in specific diseases may be perceived as more adequate by the patients and are expected to offer better sensitivity to change over time.

Of note, the self-rating of walking ability stands somewhere in between the symptom-specific PRO and the PRO of ADL, as disturbance of gait can be considered both as a clinical sign (and assessed in clinical rating) but also as an impairment of a fundamental domain of everyday functioning, related to physical activity levels, general mobility, social participation, and thereby impacting on hrQOL.

Generally, the move towards patient-centered research and cost-effectiveness research has led to increasing numbers of PRO instruments for different disorders, but also increasing standards for their development and use in clinical trials. Not least, multi-national trials will need at least linguistically validated translations when applying the same PRO in different countries. Further, practical applicability of paper-pencil or computerized PRO versions may need adaptations according to the levels of hand function impairment. Likewise, applicability in pediatric populations or those with cognitive impairment need consideration. To deal with the increasing demands on PRO, also from a regulatory perspective, overarching efforts have been put into operation to develop a framework for generalizable item banks

or common metrics, specifically for neurological disorders. One such example is the US National Institutes of Health (NIH) Patient-Reported Outcomes Measurement Information System (PROMIS) Roadmap initiative (www.nihpromis.org) (Gershon et al. 2010b). This initiative aims to provide a set of psychometrically validated PRO for several constructs of clinical relevance, that can be flexibly combined to "evaluate and monitor physical, mental, and social health in adults and children and can be used with the general population and with individuals living with chronic conditions" (www.healthmeasures.net). From the same site, a set of PRO (Neuro-QoL) is available selected for specific relevance for neurological disorders.

The following sections will shortly describe the most commonly used generic PRO of hrQOL and also describe the few PRO specific for ataxias and will mention the PRO of ADL that have been reported in large ataxia cohorts.

15.1 Generic PRO of hrQOL

The EQ-5D and the SF36 have most frequently been applied as generic instruments in ataxia studies including large observational trials (Tai et al. 2017a; Bolzan et al. 2021; Wilson et al. 2007). Although shown to reflect relevant change in impact of disease, low repeatability of EQ-VAS and low effect sizes in longitudinal observation, for example, preclude use as proxy for disease progression in longitudinal or interventional studies (Schmitz-Hübsch et al. 2010a, b; Jacobi et al. 2018). For the evolution of SF36 in FRDA, independent observations reported decline limited to SF36 physical and role limitation subscores with stable mental subscores (despite worsening in clinical ratings) (Xiong et al. 2020; Tai et al. 2017a).

Improved metric properties may be expected for the more recently devised PROMIS V1.2 general health instrument (Hays et al. 2009). It consists of 10 questions on general well-being, quality of life, bodily functioning, psychological functioning, life satisfaction, activities, social roles, mood, fatigue, and pain. While pain is rated on a 0–10 numeric scale, the remaining items are rated on five-step Likert rating. To date, no data are available on use of any PROMIS or NeurQol instrument in ataxias.

Apart from instruments that target hrQOL or activity limitations, distinct functions or symptoms may be assessed using specific questionnaires, such as balance confidence ratings (Powell and Myers 1995), questionnaire on walking function (Brogardh et al. 2021), fatigue (FSS (Krupp et al. 1989) or FSMC (Penner et al. 2009)), or instruments for sleep quality, mood, pain, or autonomic dysfunctions. Of note, some of these functions are also covered in the more recent NeuroQol but will need linguistically valid translations for international use.

15.2 PRO Specific for Ataxias

Patient-report of disease symptoms and their impact form part of the disease-specific clinical ataxia rating scales FARS (part II) and UMSARS (part II). Further, the ADL part of the Unified Rating Scale for Huntington's Disease (UHDRS part IV) has been used in large longitudinal trials in adult-onset ataxias. However, in all three scales assessment is often rater-based and obtained by structured interview and scoring thus involves some interpretation by the rater. For FARS part II, use as self-report questionnaire is preferentially applied in US sites which may lead to site differences in outcome unrelated to the disease (Reetz et al. 2021). Thus, type of assessment should be specified in the study protocols and reported along with study results.

15.3 FAIS—Friedreich's Ataxia Impact Scale

This first disease-specific PROM for application as a patient questionnaire was published in 2009 (Cano et al. 2009). It was conceptualized to assess the health impact of FRDA in clinical studies. Development of FAIS followed current methodology of scale construction including qualitative research and patient involvement to generate a conceptual framework and first item pool and Rasch measurement methods for item selection. The questionnaire consists of a 126-item long form, divided into eight subscales (speech, body movement, upper limb, complex tasks, self-perception, isolation—each with three response options—and lower limb and mood rated with four response options). As all items are scaled to a common construct, subsets of items may be selected for short forms that may better apply to specific populations, for example, more or less impaired, as proposed in the original work. Although rigorously designed, the stability of response at retest has not been established. Only some subscores showed validity against clinical ratings of ataxia (FARS) while all correlated with the SF36 mental and physical component summary scores (Tai et al. 2015b). Responsiveness was poor except for the speech subscale in this 2-year observational study while benchmarks of detectable or important change have not been established. According to current report, the scale has not seen wide application nor translations.

15.4 PROM-Ataxia

Only recently, a PRO was specifically developed for use in cerebellar ataxias, designed according to the standards of the PROMIS, "drawing on the knowledge, experience and involvement of patients throughout the process" (Schmahmann et al. 2021). The questionnaire consists of 70 items grouped into the domains of physical symptoms, the domain of activities of daily living and the domain of

mental health. Selection and weighting were based on perceived relevance, resulting in predominance of physical domain (36 items, 144 maximum score) and less weight to ADL and mental domain (each 17 items and 68 maximum score). Item ratings follow a 5-step Likert scale with zero denoting never/without any difficulty with a time-frame of 2 weeks. Item responses can be summed to above subscores as well as a total score (range: 0–280). The metric properties provided with the original publication support high scale consistency, at least substantial test-retest reliability at 15–30 days retest (almost perfect for ADL subscore) and moderate association with self-reported disability staging. Face validity was confirmed in patient focus groups as perceived importance, relevance to disease and expected responsiveness. A 10-item short form was proposed based on internal consistency measures for the full version. However, both long version and short form await proof of responsiveness from longitudinal assessment and validity testing against clinical ratings in larger and more heterogeneous cohorts.

16 Summary and Perspectives

For ataxia research, both generic ataxia rating scales for ataxias of any etiology and disease-specific scales for FRDA and MSA are currently available. Inter-rater reliability is usually reported as high with only minimal rater training. A recent review by the Movement Disorders Society Rating Scales Review Committee (Perez-Lloret et al. 2021) considered all scales as feasible in ataxia populations, but prior validation was often insufficient, specifically with respect to interpretability of score changes. Scale responsiveness is a major concern when selecting outcomes for clinical trials that aim to show effects on disease progression. Responsiveness cannot be validly determined from cross-sectional study. Prior data from longitudinal observation are of eminent importance for sample size calculations and interpretation of score changes. This implies, that feeding existing or future patient registries with scores from standard clinical ataxia ratings would be of great help to set up interventional trials, specifically for rarer ataxia conditions. Collaborative efforts and existing datasets within the ataxia global initiative (ataxia-global-initiative.net) are highly relevant in this respect.

Still, score changes in placebo arms may differ from score progression expected from natural history studies due to placebo effects. Some data are available from interventional trials that suggest that placebo effects on clinical ataxia ratings do occur but do last only for limited time-frames.

In contrast, sensitivity and specificity determined against larger appropriate healthy cohorts are of utmost importance for clinical trials that aim to delay manifestation of disease.

As a limitation specifically for early-onset ataxias, results of validation in children showed limitations of clinical ataxia rating scales below the age of 12 years for ICARS, BARS, SARA, and pegboard tests (Lawerman et al. 2017; Brandsma et al. 2014). This may also apply to other quantitative and instrumented motor tests as

well as to the bedside screen of CCAS. Age effects need further investigation before use in children and available normative datasets should be checked for applicability.

The majority of the clinical ataxia rating scales reported herein cover different domains of the cerebellar motor syndrome to different proportions and some also include other (non-ataxia) symptoms (Fig. 2), while CCAS Scale and INAS count deliberately target disease manifestations other than ataxia. Proportions per domain depicted in Fig. 2 was calculated as % of domain scores of total score (which has some uncertainty for PRO, were some items—for example "difficulty playing with children"—cannot be clearly assigned to one or the other domain). Of note, the disease-specific PRO reflects the disease impact also in other non-motor domains, such as sensory symptoms, bowel and bladder functions, sleep and fatigue, cognitive performance, affective disturbance, activities and participation. Researchers should be aware that even reliable and responsive ratings in the motor domain may miss aspects of importance to ataxia patients. The same applies to the upcoming instrumented motor testing that may evolve into motor biomarkers. This supports the recommendation for clinical trials to combine a metrically robust clinical rating or (motor)biomarker/performance measure with measures of patient perception.

Patient global impression of change may be used to classify treatment response; however, the relation of such self-report to clinical ratings or motor biomarkers needs far more exploration and cannot be assumed as linear. The perceived importance of change may differ for patients of different disability levels, may change over the individual disease course, and may not be the same for improvement or worsening according to clinical scores.

A major concern related to scale responsiveness is the score fluctuations in the short term (day-to-day variability) which hampers a reliable detection of chronic progression, i.e. score point changes. Such variability may only in part be attributed to raters, though interrater reliability is generally reported as high for clinical ratings. Rather, score variability over time may reflect within-subject variability to considerable extent, due to known and unknown factors. Recent methods of remote assessment may yield reliable estimates and determinants of such variability (Grobe-Einsler et al. 2021) to be incorporated in longitudinal data analysis. However, consensus protocols need to be established and standards of analysis from multipoint or continuous data. Also, appropriate quality control of remote data acquisition and applicable algorithms need to be defined for use in clinical studies. In sum, clinical ataxia rating scales contain educated clinical judgment as the major strength that can ensure specificity compared to timed or instrumented motor testing and can improve sensitivity compared to patient-report.

17 Future Directions for Clinical Scales

Recently, modifications of existing scales have been proposed driven by some uneasiness with existing scales as well as driven by regulatory decisions. Uneasiness with clinical rating comprises doubts on objectivity, doubtful relevance to patients,

and assumed inferiority to more reproducible quantitative measures from instrumented analysis. However, such assumptions are not supported by strong evidence and thus there is confusion what exactly a "better clinical rating scale" should look like. The plea for a higher relevance of score changes to those affected markedly contradicts the promotion of quantitative functional biomarkers that may detect even more subtle sub-clinical changes. Others proposed to reconcile different levels of assessment with the implementation of compound outcome assessments, which similarly await definition of appropriate evaluation protocols and interpretation. Applying item-response theory rather than classical test theory may prove useful also for clinical rating scales to better understand and compare their differential measurement properties and optimal range of application, as has recently been explored for clinical ratings scales in movement disorders (Chae et al. 2021; Foubert-Samier et al. 2022; Luo et al. 2021). This approach may also be explored to relate results of different instruments or components of a multi-modal assessment to model possibly differential responsiveness to change.

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Scale for Ocular Motor Disorders in Ataxia (SODA): Procedures and Basic Understanding

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Abstract The cerebellar disorders are evaluated with a number of clinical rating scales. None of these scales emphasize common ocular motor deficits. In instances when an ocular motor aspect of the disease is part of the rating scale, the subscales are limited and do not correlate with appendicular or axial components. This motivated development of a dedicated Scale for Ocular motor Disorders in Ataxia (SODA). The goal of SODA was to objectively measure the burden of ocular motor phenomenology in cerebellar disorder. SODA, like any other rating scale, does not help differentiate the etiology of the disease. This chapter outlines the summary of

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_11

SODA and provides a basic understanding of clinical assessment strategies to effectively perform ocular motor rating. We also outline mechanistic understanding, why specific aspects of ocular motor examination were included in SODA, what to expect from each of such phenomenologies, and how they are relevant to cerebellar disorders.

Keywords Rating scale · Cerebellum · Eye movements · Saccades · Nystagmus · Gaze

1 Background and Justification

Disorders of eye movements are extremely common in patients with cerebellar ataxias. Typical disorders include deficits in gaze-holding, ocular pursuit, rapid gaze shifts (saccades), or vestibulo-ocular reflex (VOR) (Leigh and Zee 2015; Kheradmand and Zee 2011; Feil et al. 2019). The eye movement disorders can be pathognomonic markers of cerebellar impairments, and they carry a potential of an objective outcome measure (see Leigh and Zee 2015). The eye movements are easy to recognize without specialized equipment and they can be monitored with objective instrumented techniques, such as video-oculography. Traditional rating scales for cerebellar ataxias, such as the Scale for Assessment and Rating of Ataxia (SARA), Spinocerebellar Ataxia Functional Index (SCAFI), and International Cooperative Ataxia Rating Scale (ICARS), lack ocular motor objective measures. Modified ICARS and Brief Ataxia Rating Scale (BARS) incorporate a short component of ocular motor assessments. The subscales of ocular motor dysfunction do not correlate with total score or appendicular and axial subscores. Consequently, it is justified to have a dedicated scale to measure ocular motor disorders in ataxias. In order to generalize its application, the scale has to be simplified to facilitate rating by non-specialized examiners; and it has to be short to incorporate in combination with other outcome measures or day-to-day clinical practice. With these goals in

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mind, a consortium of cerebellar and ocular motor experts was put together; the group generated a simple yet comprehensive ***S**cale for **O**cular motor **D**eficits in **A**taxia (**SODA**)*. The overarching aim was

1. *To establish a measure that can provide the extent of eye movement abnormalities in patients with cerebellar ataxia.*
2. *To prepare for the drug trials for symptomatic and disease-modifying effects in patients with ataxia secondary to focal/diffuse lesions of the cerebellum.*

Detailed validation of SODA and its preliminary assessment was published elsewhere (Shaikh et al. 2022). The goal of the current chapter is to outline (1) practical guidelines on how to perform different components of SODA and (2) mechanistic basis for including different components of the ocular motor and vestibular examination in SODA—why specific subgroup of the exam is involved, and what does it account for.

Table 1 depicts the summary of SODA. Then we suggest 10 basic "rules" for effective, and accurate eye movement and vestibular examination that would be beneficial for incorporation of SODA in the clinical practice. The subsequent section outlines why the specific aspect of ocular motor examination is selected, how to perform it, and what it contributes to the SODA.

Table 1 Scale for ocular motor deficits in ataxia (SODA)

Ocular alignment (1 for affirmative response, 0 when absent)	
Instruction: Examine gaze holding at distant (≥10 feet) target with each eye occluded individually	
Exotropia	
Esotropia	
Skew deviation	
SUBTOTAL	/3
Saccadic intrusions (1 for affirmative response, 0 when absent)	
Instruction: Examine gaze at straight-ahead, Right, Left, Up, and Down at 45° gaze angle using the examiner's index as target. The index finger is located about 50 cm from patient's nose. Each position for 5 seconds.	
Horizontal saccadic oscillations	
Vertical saccadic oscillations	
Square wave jerks	
SUBTOTAL	/3
Jerk nystagmus (here called "nystagmus") (1 for affirmative response, 0 when absent)	
Instruction: Examine gaze at straight-ahead, Right, Left, Up, and Down. The index finger of the examiner is used as target at a distance of about 50 cm from the patient's nose. Each position for 5 seconds.	
Spontaneous horizontal nystagmus in straight ahead gaze-holding (rebound nystagmus does not qualify)	
Spontaneous vertical nystagmus in straight ahead gaze-holding	
Sustained gaze-evoked horizontal nystagmus (no orthogonal [vertical] nystagmus) on right and/or left gaze holding position	

(continued)

Table 1 (continued)

Rebound nystagmus	
Gaze-evoked vertical downwards nystagmus on right and/or left gaze	
Gaze-evoked vertical upwards nystagmus on right and/or left gaze	
Positional nystagmus (during supine, upright, and right or left ear down testing)	
SUBTOTAL	/7
VOR Cancellation	
Ask subject to slowly move the head in no-no direction (horizontal) and yes-yes (vertical) and simultaneously ask the subject to align the gaze on target that examiner is moving with the head. While doing it look for corrective saccadic movements (1 if present, 0 if absent)	
Horizontal VOR Cancellation	
Vertical VOR Cancellation	
Ocular pursuit (1 for affirmative response, 0 when absent)	
Instruction: Ask to follow examiner's index (or bright target) that is moving slowly in front of the patient at a distance of about 50 cm	
Horizontal Saccadic pursuit	
Vertical saccadic pursuit	
Use higher subtotal value of ocular pursuit or VOR cancellation	/2
VOR	
Instruction: Perform 4 head impulses to the right and 4 head impulses to the left while fixating gaze on the distant (≥10 feet) target (1 for affirmative response, 0 when absent)	
Saccadic corrections during head impulses to the right OR left	
Saccadic corrections during head impulses to the right AND left	
Saccadic corrections during head impulses to the up OR down	
Saccadic corrections during head impulses to the up AND down	
SUBTOTAL	/4
Saccades, apraxia, and gaze restriction	
Instruction: Perform 4 horizontal saccades from center (examiners nose) to eccentric bright pointed target (or examiner's index) on the right side and on left side (2 each). Perform 4 vertical saccades from center (examiners nose) to eccentric bright pointed target down or up (2 each). For each box below, use 1 for affirmative response, 0 when absent. If gaze restriction is present, dysmetria and slowing in in that direction gest maximal point.	
Dysmetria (Hyper- or Hypo-metria) in horizontal saccades	
Dysmetria (Hyper- or Hypo-metria) in vertical saccades	
Slowing of vertical saccade	
Slowing of horizontal saccades	
Oculomotor apraxia (look for latency in initiation, NOT multiple gaze shifts to make saccade, i.e., "staircases")	
Gaze restriction vertical	
Gaze restriction horizontal	
SUBTOTAL	/7
TOTAL	**/26**

2 Ten Rules of Accurate and Effective Examination of Eye Movements and Vestibular Function

2.1 Rule 1: Assuring That the Preliminaries Are Met

It is critical to establish that the patient has acceptable visual function after correcting the refractive errors. It is ideal to examine the visual acuity using the pocket acuity card prior to examination and incorporating the findings into SODA. It is critical to perform fundus examination using ophthalmoscopy. Finally, many neurodegenerative conditions that affect the cerebellum also present with abnormal eyelid function. It is critical to assure that the lids are not closed due to blepharospasms, hemifacial spasm, or lid apraxia.

2.2 Rule 2: Organization in the Clinic, Keeping the Distance Between the Patient and the Visual Target

Many ocular motor deficits, particularly those under influence of ocular vergence, are susceptible to the viewing distance. Some types of nystagmus are dampened by convergence, while VOR may have increased gain in presence of closely located visual target. We recommend that the visual target should be 6 to −10 feet away from the patient. We typically follow the organization illustrated in Fig. 1 in the clinic setting. We ask patients to shift gaze from far target to the near target while assessing the depth and vergence dependence of some forms of nystagmus.

2.3 Rule 3: Color of the Visual Target Should Be Bright

It is recommended to use bright-colored object while examining the eye movements. Generally, the size of target is that of back of pen cap, about 5 mm in diameter. Red color avoids camouflaging the target and it can be used for red saturation test as well.

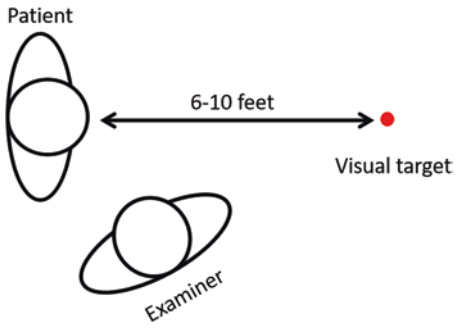

Fig. 1 Schematic organization of clinic setting. The position of the patient, examiner and the visual target

2.4 Rule 4: Ocular Alignment

Ocular alignment is an important aspect of SODA. We recommend translucent occluder for this purpose. The phenomenon of interest is the involuntary disconjugate deviation of the covered eye, but as the visual fixation is allowed, the involuntarily turned eye re-fixates on the object of interest. The translucent occluder allows visualization of the covered eyes by the examiner, while preventing patient's vision through the occluder.

2.5 Rule 5: Age-Related Changes and Effects of Medications

Cerebellar disorders are not uncommon in elderly people. Age, even in the absence of central or peripheral pathologies, can lead to atrophy, fibrosis, and restricted movements of the eyes due to changes in the orbit. Therefore, it is not uncommon for the elderly persons to have limited upward eye movements or convergence. It is essential to consider the patients' home medications while interpreting the ocular motor examination. A number of medications cause involuntary eye movements, typically antiepileptics lead to nystagmus. Some types of pharmacotherapies, especially benzodiazepines or narcotics, affect the saccade velocity.

2.6 Rule 6: Stabilize the Patient's Head

It is essential to stabilize patients' heads while performing the ocular motor examination. This is particularly critical in those with hyperkinetic movement disorders, as often seen in patients with cerebellar impairments. Even small head movements, primarily generated at the neck or transmitted from the limb or trunk, can trigger VOR. If these movements are associated with vestibular hypofunction, then it gives an impression of nystagmus, that is, "pseudonystagmus." Adequate head stabilization will rule out pseudonystagmus.

2.7 Rule 7: Pay Attention to the Eyelids

Subtle vertical eye oscillations can be diagnosed by observing the lid movements. The physiological rationale is that the vertical eye movements are yoked with the action of the levator palpebrae; every time we look up the eyelids contract and go up; and vice versa. We suggest focusing on the eyelashes or eyelids to look for subtle vertical eye oscillations of upbeat or downbeat nystagmus.

2.8 Rule 8: Use an Ophthalmoscope if Needed

Visual deficits, such as shimmering or blurred vision, can be seen with ocular flutter or micro-opsoclonus, collectively called saccadic oscillations. These deficits can also manifest as "dizziness." Examination with bare eye may not reveal microflutter or microopsoclonus, in which case it is essential to examine the eye movements with ophthalmoscope. We recommend focusing on the optic disc and the rotations of the blood vessels around the optic disc. It is also important to keep in mind that under ophthalmoscopy the eye movement direction will be switched. The reason is that the eyeballs rotate on the axis that passes from the middle of globe. The optic disc is on the other side, so the downward movement of the iris or the front of the eye, for example, is equivalent to the upward movement of the optic disc.

2.9 Rule 9: Look at the Bridge of the Nose

It is critical to notice subtle disconjugacy between the two eyes. In order to note the subtlety such as mild seesaw nystagmus, internuclear ophthalmoplegia, or dynamic disconjugacy during saccades and pursuits, we suggest looking at the bridge of the patient's nose while focusing on two eyes simultaneously.

2.10 Rule 10: Head Impulses Should Be Brief but Fast

Head impulses are important part of an examination of the VOR, an important component of SODA. The head impulses should be done in all three canal planes; horizontal, right anterior left posterior, and left anterior right posterior. In each plane, the head impulses should be fast but with brief excursions, less than 5°. The large excursions of the head impulses will render patients at the risk for developing neck pain or even worse sequels such as dissection.

3 Organizational Components of SODA

The goal of SODA is to identify an ocular motor abnormality in patients with cerebellar ataxia, while assuring it is simple enough to be utilized by nonexpert operators. We could reach this goal (Shaikh et al. 2022). This chapter will further educate the interested raters to further learn effective ways to perform SODA. The current section focuses on various aspects of SODA, how they can be performed and interpreted, and why they were included in SODA. The readers may be interested in mechanistic underpinning of various ocular motor abnormalities in the cerebellar

disease. While critical to have this knowledge, its inclusion here in this chapter will dilute the focus on SODA. Such readers are referred to the reference text, such as The Neurology of Eye Movements (Leigh and Zee 2015).

3.1 Ocular Alignment

Ocular misalignments such as exotropia, esotropia, and skew deviation are not uncommon in cerebellar disorders (Ghasia et al. 2016; Goldstein and Cogan 1961; Harris et al. 1993; Hufner et al. 2015; Khan et al. 2008; Kono et al. 2002; Rabiah et al. 1997; Wong et al. 2015). It is critical because typically any clinically identifiable ocular misalignment leads to diplopia. Ocular misalignment is often seen in people with multiple system atrophy, certain forms of spinocerebellar ataxia (e.g., SCA3), ataxia-telangiectasia, or even cerebellar strokes. Esotropia, or exotropia, or skew deviation each was assigned 1 point in SODA.

3.2 Fixation Deficits (Saccadic Intrusions)

Two types of saccadic intrusions are noteworthy—saccadic oscillations and square wave jerks. Saccadic oscillations are back-to-back saccades without intersaccadic intervals. When unidirectional they are called ocular flutter, while multidimensional saccadic oscillations are called opsoclonus. Flutter and opsoclonus are not uncommon in cerebellar syndromes due to autoimmune or degenerative process (Ghasia et al. 2016; Desai and Mitchell 2012; Ellenberger Jr. et al. 1968; Ellenberger Jr. and Netsky 1970; Helmchen et al. 2003; Hersh et al. 1994; Jen et al. 2012; Optican and Pretegiani 2017; Ross and Zeman 1967; Shaikh and Wilmot 2016; Theeranaew et al. 2021; Tuchman et al. 1989; Wong et al. 2001; Wray et al. 2011). They are commonly seen in syndrome of anti-GAD antibody, SCA3, opsoclonus-myoclonus-ataxia, and ataxia-telangiectasia (Shaikh et al. 2009; Tang and Shaikh 2019). Uniplanar fine oscillations, such as ocular flutter, are generally mild and less chaotic compared to multiplanar coarse opsoclonus. Therefore in SODA, horizontal saccadic oscillations were given 1 point, and when vertical oscillations are also present they receive another 1 point. The square wave jerks are other form of saccadic intrusions. They are frequently seen with psychiatric conditions, such as schizophrenia. Typically, they do not affect visual function, unless when they are excessive in frequency or present as entrained back-to-back square waves with increased amplitude. The classic example of cerebellar deficit causing symptomatic square waves includes spinocerebellar ataxia and saccadic intrusions (Rosini et al. 2013; Serra et al. 2008). Square waves are always horizontal; it is extremely rare to have vertical square waves. When present square waves are given 1 point on SODA.

3.3 Jerk Nystagmus

Jerk nystagmus, here called "nystagmus," features slow drifts in the position of the eyes followed by rapid (corrective) movement (i.e., the quick phase). The nystagmus in cerebellar disorders is of many types, for example, gaze-evoked nystagmus, rebound nystagmus, downbeat nystagmus, and positional nystagmus, and upbeat nystagmus (Theeranaew et al. 2021; Wray et al. 2011; Baloh and Yee 1989; Benjamin et al. 1986; Gilman et al. 1977; Higashi-Shingai et al. 2012; Kanaya et al. 1994; Kato et al. 1985; Schmidt 2011; Shin et al. 2010; Baloh and Spooner 1981; Bertholon et al. 2003; Cho et al. 2017; Choi et al. 2012, 2014; Jeong et al. 2011; Kim et al. 2013; Moon et al. 2009; Norre and Puls 1981; Sakata et al. 1987; Yabe et al. 2003). Nystagmus leads to significant impairment and is a hallmark of cerebellar dysfunction. Therefore, each form of nystagmus was considered an individual item in SODA. In each condition, when nystagmus is present, it scores 1 point; maximum score reaches 7. Subsequent section outlines each type of nystagmus, its significant in relevance to cerebellar disorders, and SODA.

3.3.1 Gaze-Evoked Nystagmus

The gaze-evoked nystagmus is the most common type of nystagmus. The eyes, when in eccentric position, drift towards the central orientation. The drift is followed by a quick phase. As a result right-ward gaze holding leads to right beating nystagmus; and left-ward gaze has left beating nystagmus. Although nystagmus is named according to the direction of the quick phase, that is, the "beat" direction, the pathognomonic aspect of the nystagmus is slow drift. The drifts are result of impaired integration of the saccade velocity command that is meant to convert it into steady state position under cerebellar feedback. Impaired cerebellar feedback in form of the cerebellar disease leads to abnormal integration and subsequently the nystagmus. As a result, the eye velocity during drift increases as the desired eccentric eye position shifts farther away from the null. As the eyes change orientation from one side of the null to the other, the drift direction also reverses. The gaze-evoked nystagmus is not only horizontal, but it can be seen in vertical direction; upbeat nystagmus in upward eye position while downbeat in downward direction. In some cases, gaze-evoked nystagmus is seen in both horizontal and vertical direction, hence eccentric gaze holding leads to oblique, or side pocket nystagmus. During central gaze, the eyes are relatively steady, but after sustained eccentric orientation, the central gaze has drifts in the direction opposite of the eccentric gaze drift. Latter phenomenon is called "rebound nystagmus." For example, after left-ward gaze holding that triggers left beating gaze-evoked nystagmus the eyes in central orientation will have right beat rebound nystagmus. Figure 2 depicts a schematic depicting the trend of gaze-evoked nystagmus. The central position is depicted with

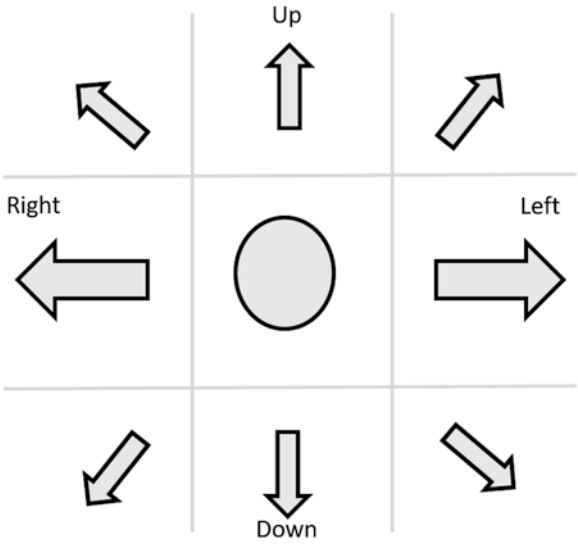

Fig. 2 Schematic depicting the ocular trajectory and intensity during gaze-evoked nystagmus. The central position with circle is when the eye velocity is null. The slow-phase eye velocity depicting drifts increase with eccentric eye in orbit orientations. Farther away orientation depicts larger slow-phase eye velocity. The size of arrow depicts larger slow-phase eye velocity. The direction of the arrow depicts the direction of eye trajectory

a circle where the eyes are stable. The arrow size in the figure depicts the intensity (the slow phase velocity) of the nystagmus, while the direction of the arrow depicts the direction of quick phase. Presence of gaze-evoked nystagmus will be scored 1 in SODA scale.

3.3.2 Downbeat Nystagmus

The downbeat nystagmus features upward drifts in the eye position followed by downward quick phase. It is the second most common form of nystagmus. Often obvious during clinical examination, in some instances one has to carefully observe the movements of eyelids to recognized downbeat nystagmus in its subtle forms. Generally, in downbeat nystagmus the eyes are relatively steady in upward gaze, but it has increased velocities of the drifts as the eye in orbit position shifts further in the downgaze. Occasionally the eye in orbit position dependence of the slow phase velocity reverses, the eye velocity is more in upgaze, and the eyes are stable in downgaze. Downbeat nystagmus is often seen with gaze-evoked nystagmus, that is, in eccentric horizontal gaze there is downbeat with left and right beat. Downbeat nystagmus is sometimes seen with headshaking, called “perverted” head shaking nystagmus. While spontaneous forms of nystagmus are part of SODA; perverted head shaking nystagmus is not considered a putative consideration in SODA. The typical trend of downbeat nystagmus is depicted in the Fig. 3, where stable eye position is illustrated with circles, while the arrow size depicts the intensity (the slow phase velocity) of the nystagmus. The direction of the arrow illustrates the direction of the quick phase. Presence of downbeat nystagmus in primary sitting position will be scored 1 in SODA scale.

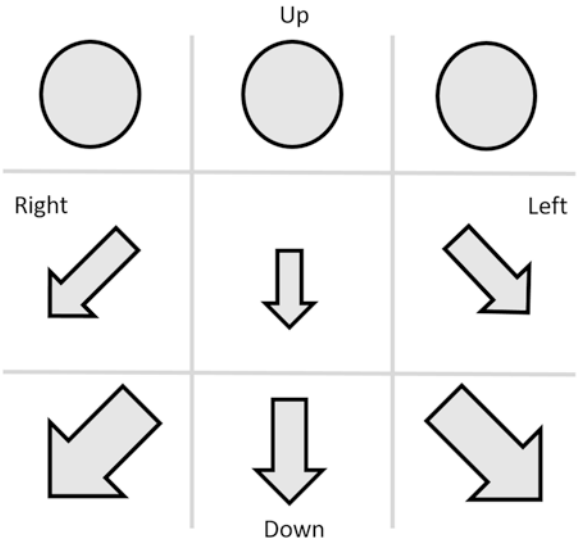

Fig. 3 Schematic depicting the ocular trajectory and intensity during downbeat nystagmus. In typical cases of downbeat nystagmus the up-gaze is steady as depicted with circle. The slow-phase eye velocity depicting drifts increase with downward eye in orbit orientations. Increasingly downward orientation depicts larger slow-phase eye velocity. The size of arrow depicts larger slow-phase eye velocity. The direction of the arrow depicts the direction of eye trajectory

3.3.3 Upbeat Nystagmus

Upbeat nystagmus is also seen in cerebellar disorders, but it is much rare compared to vertical downbeat nystagmus. Typically during upbeat nystagmus, the eyes are relatively stable in downgaze, but their slow phase velocity increases with upgaze. The drifts are downwards and beats are upwards; the eyes are stable in downgaze. The upbeat nystagmus generally suggests brainstem pathophysiology, but is also seen in with the cerebellar disorders. The upbeat nystagmus can be present as a part of gaze-evoked nystagmus when the eyes are in eccentric upgaze. Figure 4 depicts the summary of upbeat nystagmus. Here the circles are stable eye position, while the arrow size illustrate the nystagmus slow phase velocity. The quick phase direction is illustrated with the arrow direction. Presence of upbeat nystagmus during primary position will be scored 1 in SODA scale.

3.3.4 Positional Nystagmus

Positional nystagmus is not uncommon in cerebellar disorders, particularly those affecting the cerebellar nodulus. The nystagmus slow phase direction changes according to the head position, and the trend is determined by the cerebellar nodulus and ventral uvula. Often positional nystagmus can be mixed with benign paroxysmal positional vertigo, but latter has more stereotypic course and would not have accompanying movement disorders. SODA will be scored 1 if positional nystagmus is present, in any one or more head orientation, regardless of its etiology.

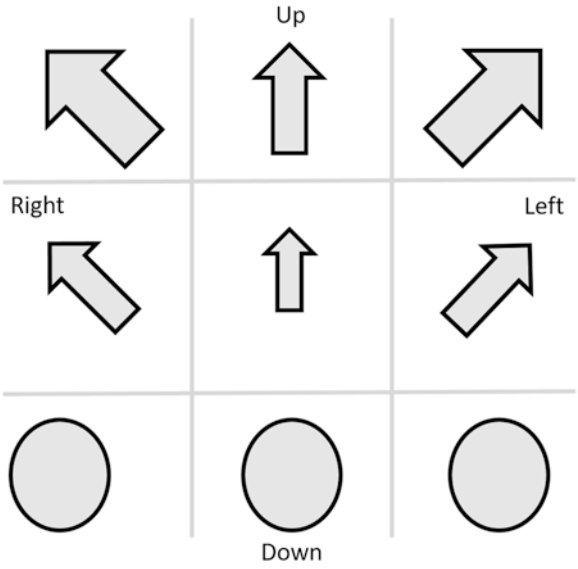

Fig. 4 Schematic illustration of the ocular trajectory and intensity during upbeat nystagmus. The downward position with circle is when the eye velocity is null. The slow-phase eye velocity depicting drifts increase with increasing upward eye-in-orbit orientations. The size of arrow depicts larger slow-phase eye velocity. The direction of the arrow depicts the direction of eye trajectory

3.4 VOR

The VOR is a critical aspect of ocular motor and vestibular examination. It is defined as a compensatory physiological eye movement in response to head movements. But it has to move at the same velocity as that of the head. It is a fundamental requirement of the VOR that the eye velocity and direction of gaze shift are precisely matched with the head velocity. Mismatch in this matric can lead to impaired visual function while locomotion. There are three ways to measure the VOR. One is the head impulse test where the head rapidly moved by the examiner in three individual canal planes—horizontal VOR, right anterior left posterior, and right posterior left anterior—vertical VOR. Another strategy includes sinusoidal oscillations of the head looking for directional and gaze disparity in VOR. Finally, the head-shaking test is a sensitive way to examine the VOR. In head-shaking nystagmus the gaze is examined in post head-shaking phase, and normally it should be stable. For simplicity, SODA only outlines most obvious test of VOR function, that is horizontal and vertical head impulse testing. The VOR hypofunction is typically considered deficit affecting the peripheral end-organs; the cerebellar dysfunction can also lead to impaired matrix of the VOR in both velocity and directional domains (i.e., perverted VOR). The impairment is secondary to inability for cerebellum to have error correction mechanism.

3.5 Saccades

The saccades are rapid eye movements made to shift gaze from one object to the other. In a given day, the humans make thousands of voluntary or involuntary saccades. Saccade size, speed, and promptness (latency) are critical to their examination. The size can be classified in larger or smaller than desired, that is, hypermetria and hypometria. Direction is measured by curved saccades, sometimes called "round the houses sign." In contrast, the velocity is much pathognomonic and depicting much worse from of cerebellar or/and brainstem disorder. Abnormal matrix of saccades depicts impaired learning and error correction mechanism that is hallmark of new cerebellar disorders.

3.6 Pursuits and VOR Cancellation

The eyes smoothly follow slowly moving target. Such eye movements, called pursuit, are examined by asking patients to follow slowly moving object in the clinic, or often the examiner's finger. Interruption in the pursuit eye movements suggests cerebellar dysfunction. In many instances of cerebellar disorders, the gaze-evoked nystagmus is present during the test of pursuit function. Latter interferes with adequate assessment of pursuit; hence, VOR-cancellation is practiced, where the moving target shifts with the head, and subject has to "cancel" the VOR to keep the eyes steady to view the target. The "cancellation" of VOR utilizes the pursuit pathway, hence impaired pursuit would lead to abnormal cancellation of VOR. SODA views pursuit and VOR cancellation as same phenomenology, and only accounts for higher of the two scores.

Acknowledgments AS was supported by philanthropic support to the Department of Neurology (Penni and Stephen Weinberg Chair in Brain Health) and the Department of Veterans Affairs Merit Review Grant (I01CX002086).

Conflict of Interest (CoI) Statement The authors have no conflict of interest pertinent to this work.

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Cerebellar Learning in the Prism Adaptation Task

Takeru Honda and Hidehiro Mizusawa

Abstract Compared with healthy subjects, patients with cerebellar degeneration find it difficult to adaptively change the movement of throwing a dart toward a virtual target image seen through a prism to that toward the actual target (prism adaptation task). This suggests that the cerebellum is related to adaptive learning. We developed a device with which anyone can perform the prism adaptation task and determine the adaptability index (AI) to estimate the capability for cerebellar learning. On the basis of basic science, it is hypothesized that the cerebellum learns internal models. In the prism adaptation task, the patients find it difficult to update either (i) the inverse model or (ii) both the forward and inverse models. Thus, the prism adaptation task can be used to estimate the capability for cerebellar learning by measuring AI. It can also be used to estimate in detail what the cerebellum learns: the forward or inverse model.

Keywords Prism adaptation task · Cerebellum · Cerebellar degeneration · Adaptability index (AI) · Internal model · Forward model · Inverse model · Adaptive learning · Cerebellar learning · Long-term depression (LTD)

1 Clinical Practice

Many conditions, such as neoplasm, trauma, congenital malformation, inflammation (including infection), immune-mediated conditions, vascular disorders, intoxication, metabolic disorders, and degeneration (Manto and Pandolfo 2002), affect

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_12

the cerebellum. Thus, for such conditions, we should evaluate the cerebellar function to assess disease severity and etiology.

In clinical practice, cerebellar signs have been detected using diagnostic methods devised by Joseph Babinski (Babinski 1899) and Gordon Holmes (Holmes 1939). Clinical scales such as the International Ataxia Rating Scale (ICARS) (Trouillas et al. 1997) and the Scale for the Assessment and Rating of Ataxia (SARA) (Schmitz-Hubsch et al. 2006) have been developed and used in routine medical examinations. Neurodegeneration is very difficult to diagnose, evaluate, and treat. For example, the SARA score is a subjective measure and depends on the experience and skill of the examiner, and its change is very subtle, only approximately one point per year in spinocerebellar ataxia type 6 (SCA 6) (Jacobi et al. 2011; Ashizawa et al. 2013; Yasui et al. 2014; Moriarty et al. 2016). Progression seems very slow in many SCA cases. A change of one point in SARA could be attributed to the placebo effect (Nishizawa et al. 2020). Therefore, a quantitative method is required to evaluate cerebellar function. This is very important for clinical trials of rare neurodegenerative diseases of the cerebellum (Manto and Pandolfo 2002).

It is considered that the factor underlying cerebellar symptoms is incoordination or coordination disorder, in which the synkinesis of muscles for performing various combinations of movements is impaired (Babinski 1899; Holmes 1939). With the latest technological development, the depth sensor Kinect v2 (from Microsoft Co.), which can measure the distance of a healthy subject's body from the sensor with infrared rays, has become available for the objective measurement of motor function of humans (Shotton et al. 2011). For example, in the finger-to-nose test, movements of not only the fingers but also the elbows and trunk can be objectively measured and compared between patients with cerebellar disease and healthy subjects (Honda et al. 2020). It is expected that such a measuring instrument will clarify the effect of coordination disorder on movement. Furthermore, it is understood that the factors underlying cerebellar symptoms are predictive movement disorder, in which an appropriate movement trajectory cannot be predicted in advance, and adaptation disorder, in which accurate movement adaptation to the surrounding environment is impaired. In the field of neurophysiology, it has been hypothesized that the cerebellum has motor learning function through synaptic plasticity (i.e., Ito 1984; Nagao 2021). There reported were some disorders of the learning function of the cerebellum as an underlying factor of the predictive movement disorder and adjustment disorder.

2 Prism Adaptation Task

A prism adaptation task has been performed by Tom Thach and his colleagues (Martin et al. 1996). When healthy subjects wore prism glasses, their field of vision was biased and they threw darts toward the virtual image of the target, making it impossible to achieve correct movement. However, by repeating dart throwing, adaptive learning occurred in response to the change in visual information, and they

could finally hit the actual target accurately. In a patient with cerebellar degeneration, even after throwing the dart repeatedly, no such learning occurs, so the patient continues to throw toward the virtual image of the target seen through the prism lens. Therefore, it is considered that the cerebellum is involved in prism adaptation.

3 Adaptability Index (AI)

In dart throwing, we must prepare a large examination room. Subjects, including patients, require some basic skills, that is, there are good and bad dart throwers. Therefore, we developed a system comprising a hand-reaching task with a touch panel so that the test can be conducted in outpatient clinics (Hashimoto et al. 2015) (Fig. 1). The system hardware consists of a personal computer (task control, data sampling and analysis), a touch screen, a pair of goggles that can fit a prism, and an ear sensor. The goggle is outfitted with an electrically controlled shutter, which opens upon applying a pulse-on command voltage (100 V) and closes on applying a pulse-off voltage. A target appears randomly at one of eight positions on the screen as the subject touches the sensor (Fig. 1a).

The goggles are connected to a sensor attached to the ear and the shutter opens when the subject touches, with the dominant hand, the sensor on the ear, or the touchscreen in front where the target is shown. As a result, just as the dart cannot be controlled after it is thrown, when the hand is released from the ear sensor, the field of vision is closed by the shutter on the goggles, and the movement of the arm follows the moment of inertia.

The actual procedure is shown in Fig. 2a. We prepared the following three sessions.

1. 50 trials with normal vision (BASELINE session)
2. 100 trials wearing prism glasses shifting the visual field 25°rightward (PRISM session)
3. 50 trials without the prism glasses (REMOVAL session)

In order to measure a large error of reaching, it might be advisable to use a prism lens that largely deviates the field of view. However, when we used the prism lens with a refraction angle larger than 25°, the touch positions were outside the 23-inch monitor of the touch panel. A healthy subject took around 20 minutes to complete the three sessions. In healthy subjects, the baseline phase showed almost no deviation; wearing the prism lens resulted in a steep deviation but soon returned to the baseline after repeated trials owing to motor learning or prism adaptation (Fig. 2a). Finally, removal of the prism caused a deviation to the opposite side because the adaptation was complete and again quickly returned to the baseline owing to the second adaptation. In a SCA31 patient, the pattern was not normal even in the baseline phase, which is indicative of dysmetria (Fig. 2b).

We have developed AI values quantified the adaptive learning function which are collected as stable data by using touch sensor technology to change the prism

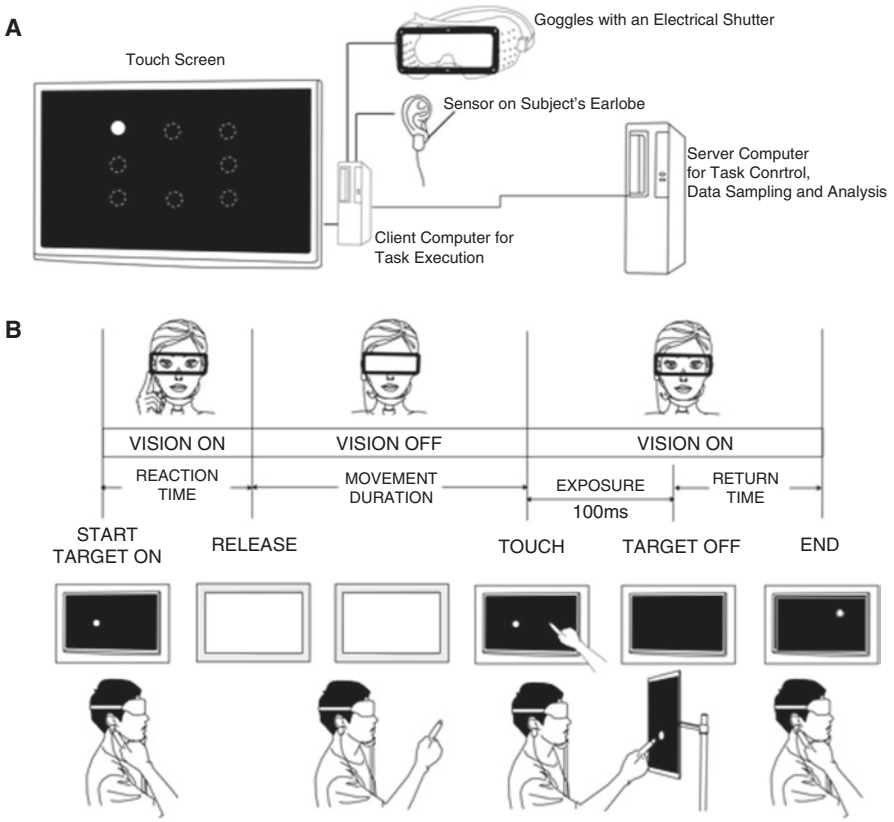


Fig. 1 Scheme for prism adaptation of hand-reaching. These figures were modified from those in our previous report (Hashimoto et al. 2015). (**a**) The system used in experiments consists of a sensor on the participant's right earlobe, goggles equipped with an electrically controlled shutter with a plastic or Fresnel prism plate, a touchscreen, and two computers. (**b**) Time sequence of single trial shown from left to right. Each trial starts from the time the subject touches the sensor on the right earlobe with the index finger. As soon as the subject releases their index finger from the sensor, vision is blocked by the shutter (MOVEMENT TIME). Immediately after touching the touchscreen (TOUCH), the goggles become transparent, and the subject can see how their index finger deviated from/hit the target for 100 ms (EXPOSURE). Subsequently, the target disappears (TARGET OFF) and the subject returns their index finger to the original position in preparation for the next trial

adaptation task from throwing darts to the reaching movement of the hand (Hashimoto et al. 2015). This has made it possible to objectively measure motor learning functions involving the cerebellum. From the data of healthy subjects, each trial was classified into one of two outcomes, "successful" and "unsuccessful." "Success" here means that there is an error of 25 mm or less from the center of the target (Fig. 2a). We define *a*, *b*, and *c* as the number of successes in the final 10 trials "with prism" (acquisition), the number of successes during the 5 trials of starting "without prism" (retention), and the number of successes in the final 10 trials

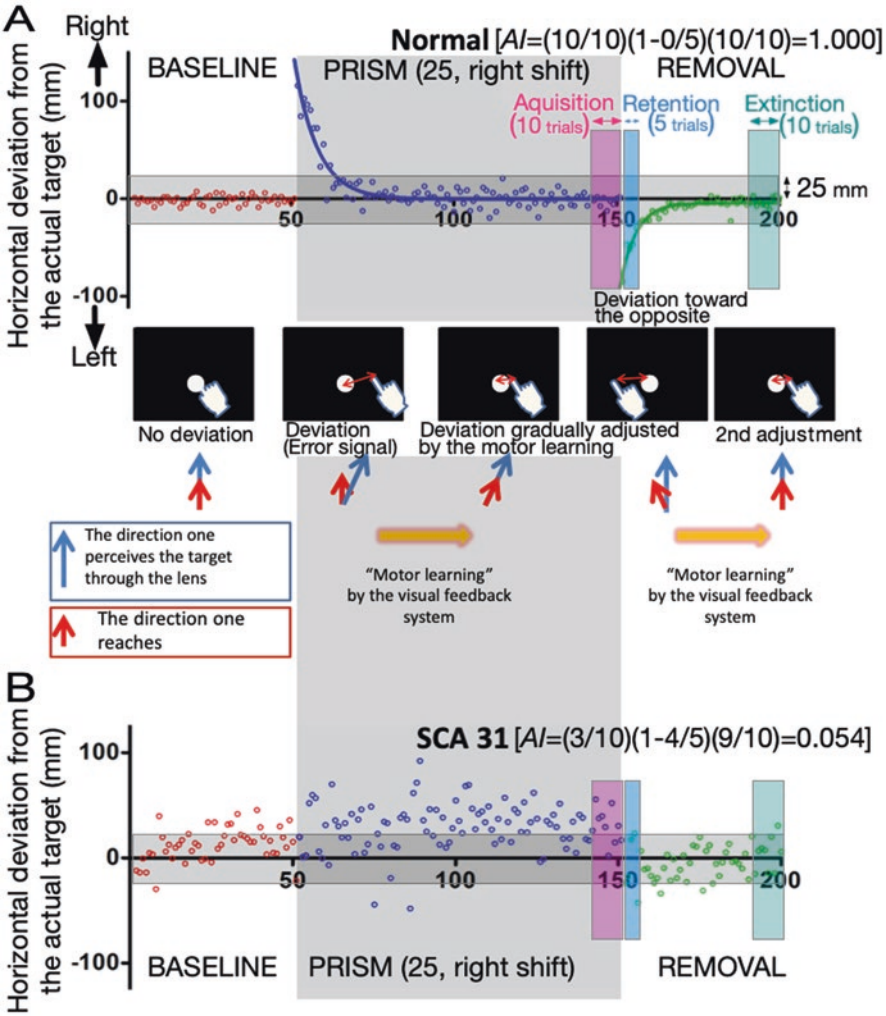


Fig. 2 Adaptation curves for a healthy subject (**a**) and a patient with SCA31 (**b**). The ordinate shows the finger-touch error represented by the distance (mm) from the target to the touch point. Positive values indicate rightward shifts and negative values indicate leftward shifts. The abscissa shows the trial numbers. Best-fitted exponential curves are overlaid on the raw data

"without prism" (extinction), respectively. From these values, the AI that takes a value of 0 to 1 was obtained using the formula $AI = a \times (1 - b) \times c$ (Fig. 2). Whereas a healthy subject had $AI = 1.000 \times (1 - 0.000) \times 1.000 = 1.000$ (Fig. 2a), a SCA31 patient had $AI = 0.300 \times (1 - 0.800) \times 0.900 = 0.054$ (Fig. 2b).

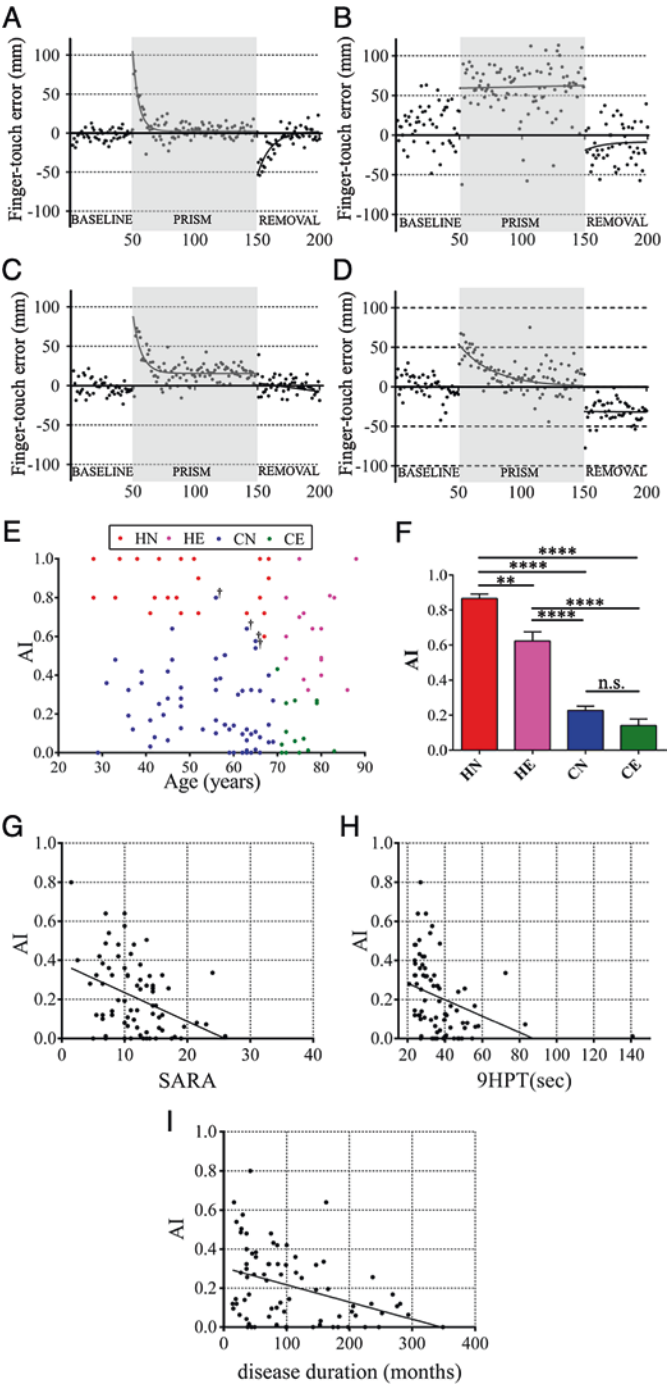
Adding the type of healthy subjects (Fig. 3a, $AI = 1.000$), we categorized the patients with cerebellar degeneration in accordance with their type of behavior in the prism adaptation task. One group showed low values of a, suggesting an

abnormality in memory acquisition (Fig. 3b, $AI = 0.120$). The second group showed high a and c values but low b values, suggesting an abnormality in memory retention (Fig. 3c, $AI = 0.000$). The third group showed high a and b values but low c values, indicating an abnormality in memory extinction (Fig. 3d, $AI = 0.144$).

The actual AI and age of each patient are shown in Fig. 3e (Hashimoto et al. 2015). Older subjects had significantly lower AI than younger subjects (Fig. 3f). When the AI was compared between patients with cerebellar degeneration and healthy subjects, there was very little overlap (Fig. 3e), suggesting that AI could be used to distinguish patients with cerebellar degeneration from healthy subjects. Indeed, AI below 0.68 had a sensitivity of 98.4% and a specificity of 100% for healthy subjects and patients with cerebellar degeneration (Hashimoto et al. 2015). AI values of patients with high SARA scores (Fig. 3g) or long time in the 9-hole peg test (9HPT) (Fig. 3h), are lower than the cut-off level. As expected, AI progressively decreased with the duration of the disease (Fig. 3i).

Until now, there has been no biomarker that can be applied to early-stage spinocerebellar degeneration and it is difficult to judge the therapeutic effect on the basis of objective evaluation indexes. Therefore, no fundamental treatment method has been developed for various types of spinocerebellar degeneration. However, it is important to detect minute changes sensitively in clinical practice, and it is expected that objective evaluation indicators such as AI will lead to early detection of such degeneration. As an example, together with this AI, it will be possible to investigate the relationship between motor learning function and the location of cerebellar atrophy caused by a disease from cerebellar volume measurement by MRI (Voxel-Based Morphometry). We found that AI is significantly correlated with cerebellar hemispheric atrophy in the right lobule VI and the left Crus I in the cerebellum, suggesting that these areas are involved in adaptive learning in the prism adaptation task (Bando et al. 2019). It is expected that further knowledge will be accumulated and AI will be utilized for early diagnosis and clinical trials. Moreover, in order to relationship between learning function and the ataxia severity, we introduce findings in basic science for the cerebellum.

Fig. 3 Adaptation curves for different subjects in healthy and patient groups. These figures were modified from those in our previous report (Hashimoto et al. 2015). (**a**–**d**) Adaptation curves for a healthy subject (**a**), patients with SCA6 (**b**, **c**), and a patient with SCA31 (**d**). The ordinate shows the finger-touch error represented by the distance (mm) from the target to the touch point. Positive values indicate rightward shifts and negative values indicate leftward shifts. The abscissa shows the trial numbers. Best-fitted exponential curves are overlaid on the raw data. Whereas a normal subject shows typical adaptation (**a**), patients with cerebellar diseases show three different patterns of impaired adaptation (**b**–**d**). (**e**) Distribution of AI values and ages for all the subjects analyzed. AI tended to decrease and showed a widespread distribution in a group of the elderly healthy subjects (HE) 70 years old and over. Cerebellar patients (CN who are under 70 years old and CE who are 70 years old and over) showed lower AI values than the age-matched healthy subjects (HN and HE). † indicates four pure parkinsonian MSA patients without clinical cerebellar signs. (**f**) Comparison of AI among the HN, HE, CN, and CE groups. In all panels, red circles and columns represent HN; magenta, HE; blue, CN; and green, CE. $^{**}p < 0.01$, $^{****}p < 0.0001$, Kruskal–Wallis test or Steel–Dwass test. Error bar represents SEM. (**g**–**i**) Scatter plots of AI and SARA scores (**g**), 9–hole peg test (**h**), and disease duration (**i**) in CN and CE patients. Linear regression lines are overlaid


A
B
C
D
Finger-touch error (mm)
BASELINE
PRISM
REMOVAL
E
HN
HE
CN
CE
AI
Age (years)
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****
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****
****
n.s.
HN
HE
CN
CE
G
SARA
H
9HPT(sec)
I
disease duration (months)

4 Findings in Basic Science for the Cerebellum

What is the mechanism of cerebellar learning? In 1967, a book entitled "The Cerebellum as a Neuronal Machine" (Eccles et al. 1967) was published, which revealed the circuit structure of the cerebellum. Immediately after its publication, the next question was on how the cerebellum functions with its simple structure, which remains a frontier theme more than half a century later (Honda and Ito 2017).

Discussion on this matter subsequently influenced brain science. In 1969, David Marr proposed the existence of synaptic plasticity in which the signals from climbing fibers elicit long-term synaptic transmission between parallel fibers and Purkinje cells in the cerebellum. Additionally, he proposed the hypothesis that the cerebellum functions as a learning machine owing to this synaptic plasticity (Fig. 4a) (Marr 1969). Furthermore, James S. Albus proposed the idea that the cerebellum is equivalent to the perceptron, which is the basis of modern artificial intelligence and deep learning (Fig. 4a) (Albus 1971). In particular, Albus predicted that synaptic plasticity would be due to long-term depression (LTD), which is the persistent attenuation of synaptic transmission efficiency. In 1982, Masao Ito and his colleagues discovered LTD and demonstrated its existence brilliantly (Ito et al. 1982; Ito and Kano 1982), proving that the cerebellum is indeed a learning machine. Masao Ito also discovered that the cerebellum is involved in adaptive learning in the vestibulo-ocular reflex (VOR), and he proposed the flocculus hypothesis, that is, this adaptive learning function is derived from LTD (Ito 1970, 1972, 1974, 1984). Together with the cerebellar perceptron model, the Marr–Albus–Ito hypothesis was established, on the basis of which the adaptive filter (Fujita 1982) and liquid state machine (Yamazaki and Tanaka 2007; Honda et al. 2011) were proposed (Fig. 4b).

5 Internal Models in the Cerebellum

The next question is "what is learned in the prism adaptation task." According to the British psychologist Kenneth Craik, "If you have an internal model as a small model of the reality of the outside world and the actions you can take in your head, you can experiment with different options (Craik 1943). And you know which option is best before you act." In 1970, Masao Ito thought that there was an internal model in the cerebellum and focused on the cerebral–cerebellar loop discovered by Peter L. Strick and his colleagues (Dum and Strick 2003; Kelly and Strick 2003). The internal model provided by Masao Ito is a forward model that predicts the sensory consequences of a performed movement (Ito 1970). In 1987, Mitsuo Kawato proposed an inverse model that predicts the motor commands that generate an appropriate sensory consequence (Kawato et al. 1987). The question we addressed in our studies on the functions of forward versus inverse models in human motor learning has been unresolved for around 35 years (Kobayashi et al. 1998; Winkelman and Frens 2006; Ebner and Pasalar 2008; Ebner et al. 2011).

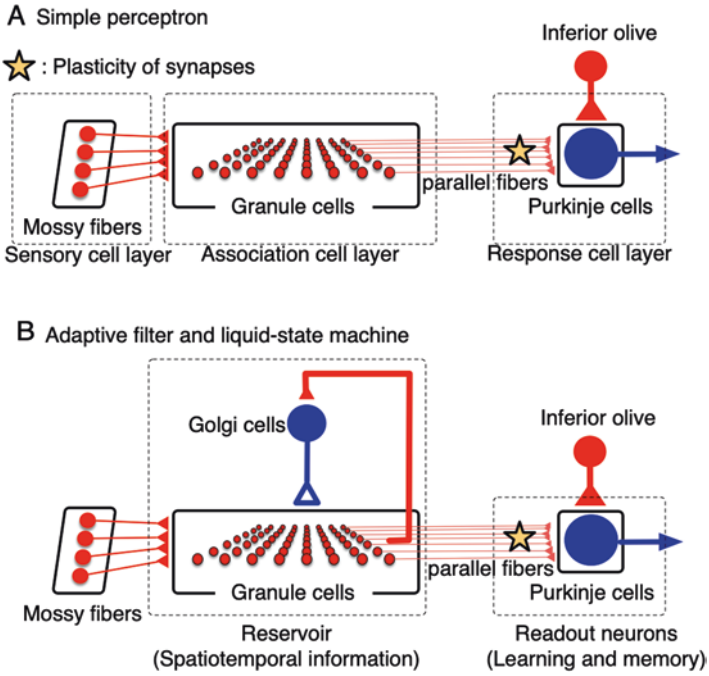

Fig. 4 Computational neural network model for the cerebellum. (**a**) Simple perceptron model consisting of three layers: sensory cell layer (containing mossy fibers), association cell layer (granule cells), and response cell layer (Purkinje cells). (**b**) Adaptive filter and liquid state machine models consisting of reservoir (granule and Golgi cells) and readout (Purkinje cells). Climbing fibers function as an external teacher in both of these models. Mossy and climbing fibers and granule cells output excitatory signals. Golgi and Purkinje cells output inhibitory signals

6 Tandem Internal Models

It is possible to determine the internal models with which the cerebellum learns by investigating the conditions that elicit such learning. A healthy subject tried to reach toward a target in the prism adaptation task under the condition that the shutter did not open even if he/she touched the touch panel on which a target appeared (non-feedback task) (Fig. 5). Under this condition, he/she could not confirm the target position and the touch position (red point in Fig. 6a) (Honda et al. 2018). When looking at the target through the prism lens, he/she touched the virtual image seen through the prism lens, so he/she could not accurately touch the target (Fig. 6a, the first 10 red dots). In other words, it is possible for an examiner to determine where the virtual image that the subject is looking at is on the touch panel. Next, we set the shutter to open when the subject touched the touch panel. Namely, he/she could see the target position and the touch position when he/she touched the touch panel and could modify his/her reaching movements (aimed offline-feedback task) (Fig. 6a, 100 black dots). Under this condition, learning occurred and he/she could touch the

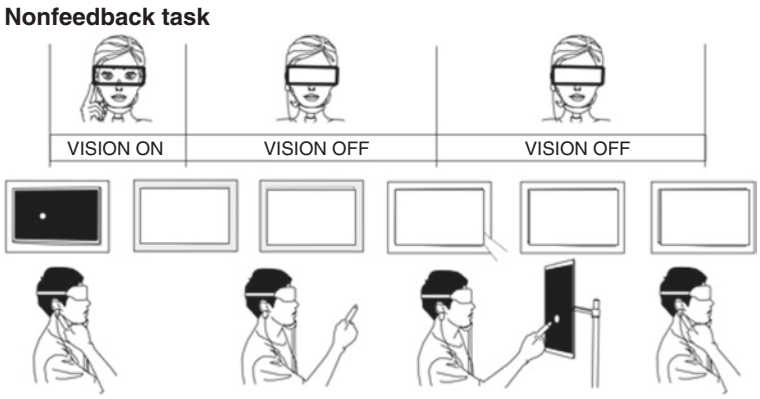

Fig. 5 Scheme for prism adaptation of hand-reaching in the nonfeedback task. Compared with the offline-feedback task shown in Fig. 1b, subjects cannot confirm the position of a target and the point touched

target accurately. After that, we returned to the condition of the nonfeedback task where the shutter did not open even when the subject touched the touch panel on which the target was displayed. Although he/she could not confirm the target position and the touch position, he/she could touch the target accurately unlike before learning (Fig. 6a, the last10 red dots). Therefore, the visual information on the target position and the touch position led to learning, so that the internal model of the cerebellum was updated from the movement information about touching the virtual image to the motion information about touching the target.

Next, although a healthy subject could confirm the target position and the touch position when he/she touched the touch panel, he/she attempted to reach and touch not the real target but the virtual image of the target (blue points in Fig. 6b) (non-aimed offline-feedback task). After that, to investigate whether the internal model of the cerebellum changes, the subject performed a task in which the reaching movement was repeated under the condition of the nonfeedback task, that is he/she could

Fig. 6 (continued) the non-aimed offline-feedback task (blue lines) followed by 10 trials of the aimed offline-feedback task (black lines). (**d**) As in (**c**) but for the nonfeedback task (red lines). (**e**) Learning operation for the tandem internal models in prism adaptation. Control system model for the aimed offline-feedback task. The switch is operated in accordance with Instruction 3 (Ins 3). Red lines represent the feedforward circuit including the inverse model. Green lines represent the internal feedback circuit including the forward model. Instruction 1 (Ins 1) represents positions of the target point before reaching movement. Instruction 2 (Ins 2) represents visual error signals, which activate learning processes in both the forward and inverse models via climbing fibers in the cerebellum. Ins 3 is generated from a high center and mediates the psychophysical command, "Do not learn from either motor or visual errors in determining the index finger's position." (**f**) Scatter diagram of Ifast vs Islow for healthy subjects and cerebellar patients. Patients in case 3 (blue squares) tended to have higher SARA scores than those in case 2 (red triangles). (**g** and **h**) Averaged adaptation curves for cerebellar patients with SCA6 and SCA31 indicating Islow <0.5 and Ifast ≥0.5 (**g**) or Islow <0.5 and Ifast <0.5 (**h**). *a*, *b*, and *c* show the periods of 10 trials

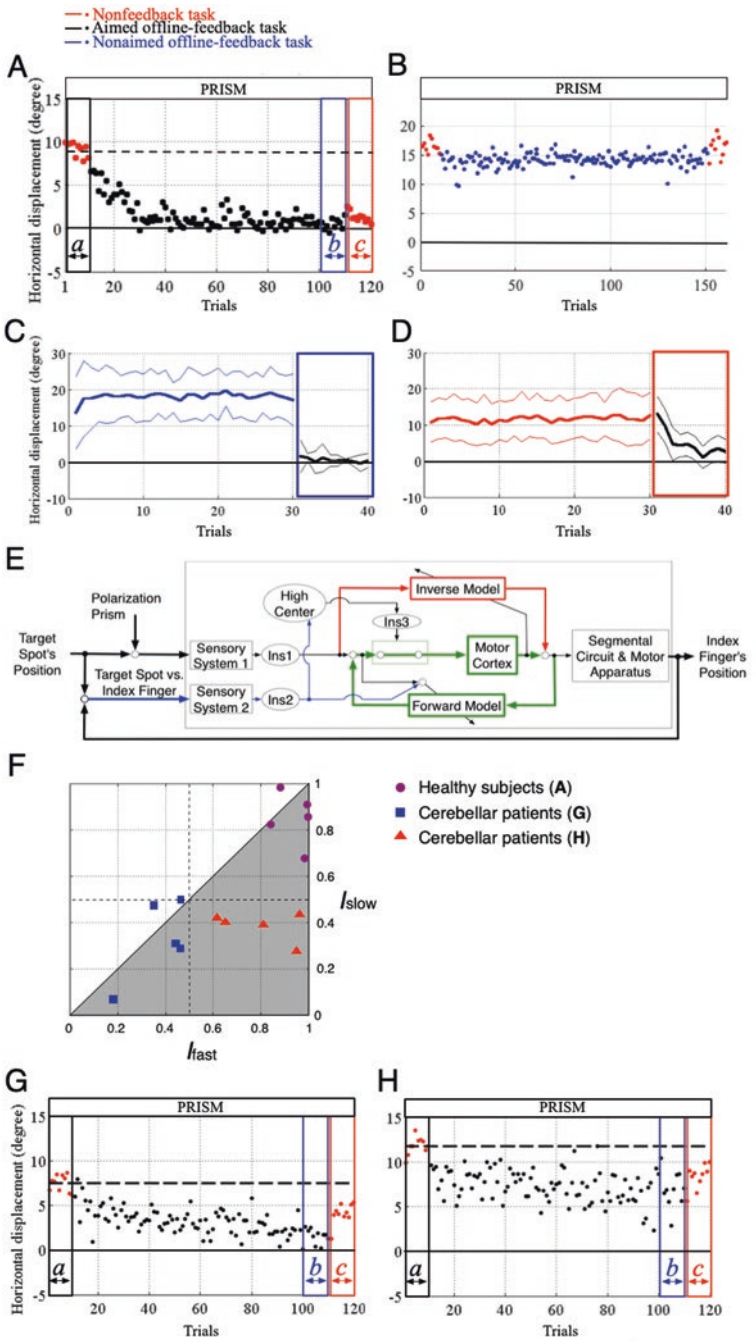

Fig. 6 Experiments and theory for the tandem internal models. These figures were modified from those in our previous report (Honda et al. 2018). (**a**) Average of five healthy subjects. (**b**) Nonaimed offline-feedback task (blue dots) and nonfeedback task (red dots) tasks. (**c**) Thirty trials of

not confirm the target position and the touch position. In this task, the virtual image was still touched, so that no changes were shown (blue dots in Fig. 6b). Even when neither the target position nor the touch position could not be confirmed (the non-feedback task), the virtual image was repeatedly touched (red dots in Fig. 6b). This suggests that this internal model in the cerebellum cannot be updated without realizing the correct movement by which the subject precisely touched not the virtual image of the target but the real target. Because the correct movement elicit updating this internal model, this internal model is the inverse model (Table 1).

Finally, under the condition of the non-aimed offline-feedback task in which a healthy subject could confirm the target position and the touch position when he/she touched the touch panel, he/she attempted to reach and touch the virtual image of the target (blue line in Fig. 6c). The subject repeated this in 30 trials, after which, he/she attempted to reach and touch not the virtual image of the target but the real target (aimed offline-feedback task). Surprisingly, the subject could touch the target correctly from the first trial (black line in Fig. 6c). On the other hand, after 30 trials in the nonfeedback task, the subject could not easily touch the target accurately in 10 trials in the aimed offline-feedback task (black line in Fig. 6d). It is considered that this internal model of the cerebellum learns the error between the positions of the virtual image of the target and the real target separately from the inverse model. That is, the result of motion (motion prediction) is learned. This is called the forward model (Table 1).

The internal model of the cerebellum that can be learned becomes clear by controlling the feedback information as described above, and a theoretical framework can be constructed (Fig. 6e) (Honda et al. 2018). In this study, we found that both the forward model and the inverse model exist. These results suggest that the forward model is updated by learning from the error signal, so that the correct motion can be voluntarily performed. The results also suggest that the inverse model is updated by realizing the correct motion. Because the inverse model is updated appropriately, healthy subjects can unconsciously realize the correct motion. Therefore, since it is considered that the forward model and the inverse model are connected in series of learning, they are called tandem internal models, that is, the learning is called tandem learning.

Table 1 Conditions of updating internal models

	Aimed offline-feedback task		
	1st to around 40th trial (Error)	Around 40th to 100th trial (Correct movement)	Non-aimed offline-feedback task (Error)
Inverse model	△	Learned	×
Forward model	Learned	△	Learned

Healthy subjects confirmed the error between the target and their touch position from first trial to around 40th trial in the aimed offline-feedback task (Fig. 6a). After around 40th trial in the aimed offline-feedback task, they precisely touched the target (correct movement) and did not confirm the error. In the non-aimed offline-feedback task, they touched virtual target and confirmed the error

7 Indexes for Tandem Internal Model

It was expected from the theory of tandem learning that if the ability of the forward model to learn declines, the learning ability of the inverse model also declines (gray area in Fig. 6f). This suggests that there is no case where the inverse model is updated, while the forward model is not updated (Honda et al. 2018). We actually measured the learning ability of healthy subjects and patients with various types of spinocerebellar degeneration using the slow adaptation index defined as I_{slow} = 1 − (hand-reaching errors during period *c*)/(hand-reaching errors during period *a*) and the fast adaptation index defined as I_{fast} = 1 − (hand-reaching errors during period *b*)/(hand-reaching errors during period *a*). The results are shown in Fig. 6a, g, and h (Honda et al. 2018). We found case A where healthy subjects could update both forward and inverse models (Fig. 6a, purple circles in Fig. 6f), case B where patients could update the forward model but not the inverse model (Fig. 6g, red triangles in Fig. 6f), and case C where patients could update neither the forward model nor the inverse model (Fig. 6h, blue squares in Fig. 6f). Patients in case C tended to have higher SARA scores than those in case B, suggesting that patients in case C have severe cerebellar symptoms. Thus, it is possible to determine in detail the learning ability of the forward and inverse models through the theory of tandem learning in detail.

8 Summary/Importance of Collaboration Between Clinicians and Basic Scientists

We developed a system with which anyone can perform the prism adaptation task and determine the adaptability index (AI) to estimate the capability for cerebellar learning. Furthermore, by using the prism adaptation task, we found that the healthy cerebellum learns both the forward and inverse models. The patients with cerebellar degenerations find it difficult to update either (i) the inverse model or (ii) both the forward and inverse models. Thus, the prism adaptation task can be used to estimate the capability for cerebellar learning by measuring AI and in detail what the cerebellum learns: the forward or inverse model.

In this article, we propose the use of AI for the evaluation of learning function in prism adaptation in addition to classical symptom evaluation. To disseminate the use of this index for evaluations, we are promoting integrated efforts of basic science and clinical practice. In Japan, Itsuro Sobue and others investigated the efficacy of thyrotropin-releasing hormone (TRH) for treating spinocerebellar degeneration ataxia (Sobue et al. 1983). Basic scientists including Masao Ito had joined this project and collaborated with clinicians to elucidate the mechanism underlying its medicinal effect (Ito et al. 1986). At present, after around 40 years of studying TRH, we continue to research through international collaboration between clinicians and basic scientists more often in order to understand the healthy

cerebellum and the mechanisms that lead to cerebellar diseases, which will contribute to develop therapies that have been proven to be effective in the prevention, treatment, and cure of the many patients and families all over the world suffering from cerebellar ataxia.

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Blood and CSF Biomarkers in Autosomal Dominant Cerebellar Ataxias

Giulia Coarelli and Alexandra Durr

Abstract A biomarker can be defined as a measurable indicator of the presence or severity of a disease state, often present before clinical signs are evident. For the most frequent forms of spinocerebellar ataxia (SCAs), due to expansions of coding CAG repeats SCA1/*ATXN1*, SCA2/*ATXN2*, SCA3/*ATXN3*, SCA6/*CACNA1A*, SCA7/*ATXN7*, SCA17/*TBP,* and DRPLA/*ATN1,* gene therapies are planned. Reliable biomarkers should indicate the pathological onset or discriminate disease stages that would allow to stratify patients and to monitor drug efficacy. This chapter reviews the available blood and cerebrospinal fluid (CSF) biomarkers. One of the most promising biomarkers is neurofilament light chain (NfL) for which blood and CSF levels accurately correlate. Moreover, NfL concentrations are associated with disease progression, and cerebellum and brainstem atrophy. Specific ataxin bioassays are in development for polyglutamine SCAs, but only ataxin-3 can be measured in blood and CSF. Other biomarkers are related to oxidative stress, inflammation, astrogliosis, and insulin pathway. Others are in development regarding the metabolism of cholesterol, lipids, and amino acids, as well as the microRNAs that would be potential biological markers of disease and therapeutic targets.

Keywords Spinocerebellar ataxias · SCAs · Biomarkers · Gene therapy

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_13

1 Introduction

Autosomal dominant cerebellar ataxias (ADCAs) are a rare cause of cerebellar ataxias. In genetic nomenclature, they referred to spinocerebellar ataxias (SCAs), a group of diseases clinically and genetically heterogeneous (Klockgether et al. 2019). Nowadays, 48 SCAs subtypes have been identified. The most frequent SCAs are due to pathological CAG repeat expansions coding for polyglutamine (polyQ): SCA1/*ATXN1*, SCA2/*ATXN2*, SCA3/*ATXN3*, SCA6/*CACNA1A*, SCA7/*ATXN7*, SCA17/*TBP* and DRPLA/*ATN1*. Age at onset and disease severity are negatively correlated with the pathological CAG repeat expansion (Durr 2010), and phenotype is clearly associated with CAG repeat size (Stevanin et al. 2000). Pediatric and juvenile forms can also occur, especially for SCA2 and SCA7 (Mao et al. 2002; Bah et al. 2020). PolyQ subtypes clinically share the cerebellar ataxia with gait and balance impairment, limb dysmetria, dysarthria, swallowing difficulties, and oculomotor abnormalities. However, other extra-cerebellar signs are also present: pyramidal syndrome for SCA1, SCA3, SCA7, SCA17, parkinsonism for SCA2, SCA3, SCA7, fasciculations and wasting for SCA2, peripheral neuropathy for SCA2 and SCA3, dystonia for SCA2, SCA3, SCA7, SCA17, choreic movements for SCA17, ophthalmological deficit for SCA7, etc. The Scale for the Assessment and Rating of Ataxia (SARA) (Schmitz-Hübsch et al. 2006), which includes eight items to assess cerebellar syndrome, does not catch these extra-cerebellar signs. This scale is used as the primary outcome in several therapeutic and non-therapeutic trials for SCAs. However, presymptomatic carriers, defined by a SARA score <3 out of 40, can present other non-cerebellar signs and symptoms that are already expression of disease.

Individual variability, even among genetically homogeneous forms due to a same mutation, impedes prediction of progression of the imaging and clinical signs in ataxias. Broadly, a higher number of CAG repeats within the *HTT* gene predicts earlier onset, but two people with the same repeat length may differ in clinical onset by decades (Lee et al. 2012). This variability has to be tackled using biomarkers that allow to define the state of disease for a single patient and the challenge for the evaluation of potential treatments, particularly in early stages, will rely on longitudinal biomarkers.

Gene therapies have made remarkable progress over the last decade, such as antisense oligonucleotides (ASOs) approach. These are targeted treatment based on the genetic status. The rationale relies on the fact that lowering the burden of mutated protein may improve the disease prognosis. ASOs form a complex with targeted mRNA recruiting an endoribonuclease (Ribonuclease H) that degrades the RNA-DNA hybrid complex (Wild and Tabrizi 2017). Following the impressive results of nusinersen in spinal muscular atrophy (Finkel et al. 2017; Acsadi et al. 2021), major hopes have been put in the development of ASO directed to *ATXN1*, *ATXN2*, *ATXN3,* and *ATXN7* mutants even though one recent phase-III clinical trial failed to show that ASOs halted the progression of Huntington disease (HD) (Tabrizi et al. 2019; Kingwell 2021). Promising results have been reported by the ASOs administration in several SCAs mouse models (Friedrich et al. 2018; Scoles et al. 2017; McLoughlin et al. 2018; Niu et al. 2018). Therefore, objective and quantitative biomarkers rather

than clinical measures are of critical importance as prognostic or pharmacodynamic markers to monitor drug effects. The aim of this chapter is to review the blood and cerebrospinal fluid (CSF) biomarkers for SCAs (Table 1).

2 Biological Biomarkers

2.1 Neurofilament Light Chain

Neurofilament light chain (NfL) is a subunit of neuronal cytoskeleton and its level increases in CSF and blood as a result of axonal damage due to different causes (neurodegeneration, infection, traumatic, etc.) (Gaetani et al. 2019). Using the highly sensitive single-molecule array method (Simoa) is possible to measure NfL in blood and CSF more accurately than conventional enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence-based method (ECL assay) (Kuhle et al. 2016). Close correlation exists between CSF and blood concentrations, making NfL an easily measurable biomarker of neurodegeneration (Gaetani et al. 2019; Khalil 2018). In several neurological disorders, NfL correlates with disease stages, clinical scores, and neuroimaging data. It is the case for amyotrophic lateral sclerosis (ALS) (Lu et al. 2015; Benatar et al. 2018), Alzheimer's disease (Mattsson et al. 2019; Benedet et al. 2020; Preische et al. 2019), and multiple sclerosis (Bjornevik et al. 2020; Kuhle et al. 2019), HD (Byrne et al. 2017; Johnson et al. 2018; Scahill et al. 2020). This latter disease shares with SCAs the same mutational mechanism, a translated pathological CAG repeat expansion. NfL showed a prognostic value with a significant increase for HD presymptomatic carriers of the pathological expansion close to the age at expected disease onset (Scahill et al. 2020). Moreover, for HD, atrophy of cerebral regions, as the putamen and caudate, is associated with higher NfL concentrations (Scahill et al. 2020).

For SCAs, some studies showed the higher NfL levels in carriers than healthy controls. The first pilot study included only 20 SCAs carriers (SCA1, SCA2, SCA3, SCA6) founding elevated Nfl concentrations compared to controls (Wilke et al. 2018). Then, other studies on large cohorts of SCA3 carriers confirmed the correlation between clinical progression and NfL in CSF (Li et al. 2019) and blood (Li et al. 2019; Wilke et al. 2020; Peng et al. 2020). In a longitudinal study with 2-year interval of plasma NfL measurements, NfL confirmed to be a disease biomarker with significant difference between healthy controls (~10 pg/mL) and polyglutamine (polyQ) SCAs carriers [SCA1 (~24 pg/mL), SCA2 (~20 pg/mL), SCA3 (~35 pg/mL), and SCA7 (~26 pg/mL)] (Coarelli et al. 2021). Interestingly, NfL concentrations remained stable at 2-year follow-up despite clinical progression assessed by SARA (Coarelli et al. 2021). Considering all SCAs subtypes, higher plasma NfL levels at baseline predicted a higher SARA score progression as well as a decrease in cerebellar volume at 2-year follow-up (Coarelli et al. 2021). NfL correlated with pons atrophy at baseline and follow-up (Coarelli et al. 2021), confirmed for SCA3 group taken separately. For SCA3, another study also reported significant

Table 1 Blood and cerebrospinal fluid biomarkers in spinocerebellar ataxias

Mechanism	Biomarker	Results	Correlations
Neuroaxonal damage	NfL	↑ in serum SCA1, SCA2, SCA3, SCA6 (Wilke et al. 2018) ↑ in plasma SCA1, SCA2, SCA3, SCA7 (Coarelli et al. 2021) ↑ in plasma and CSF of pre-symptomatic and symptomatic SCA3 (Li et al. 2019) ↑ in serum of symptomatic SCA3 (Wilke et al. 2020)	For SCA3: disease stage (Li et al. 2019; Wilke et al. 2020), CAG (Wilke et al. 2020), SARA (Li et al. 2019; Wilke et al. 2020; Coarelli et al. 2021), CCFS (Coarelli et al. 2021), pons atrophy (Coarelli et al. 2021), cerebellum and brainstem atrophy (Li et al. 2019) For polyQ SCAs: SARA, CCFS, pons atrophy (Coarelli et al. 2021); disease stage, disease duration, SARA (Shin et al. 2021)
	Phosphorylated neurofilament heavy chain	↑ in serum of SCA3 (Wilke et al. 2020)	
	Tau	↑ in CSF of SCA2 (Brouillette et al. 2015)	No
Astricitosis and gliosis	Neuron-specific enolase	↑ in serum of SCA3 (Zhou et al. 2011)	Disease duration, ICARS, and SARA (Zhou et al. 2011)
	S100B	↑ in serum of SCA3 (Zhou et al. 2011)	No
ATXN-3 bioassays	Expanded polyQ ATXN-3	Detection in PBMC by TR-FRET immunoassay in pre-symptomatic and symptomatic SCA3 (Gonsior et al. 2020)	Disease stage and SARA (Gonsior et al. 2020)
		Detection in plasma and CSF by electrochemiluminescence immunoassay in pre-symptomatic and symptomatic SCA3 (Prudencio et al. 2020)	No
		Detection in plasma and CSF by single molecule counting in pre-symptomatic and symptomatic SCA3 (Hübener-Schmid et al. 2021)	Age at onset (negative correlations) and SARA for plasma ATXN-3 levels (Hübener-Schmid et al. 2021)

(continued)

Table 1 (continued)

Mechanism	Biomarker	Results	Correlations
Oxidative stress	Superoxide dismutase	↓ in serum of symptomatic than in pre-symptomatic SCA3 carriers (de Assis et al. 2017)	No
	Glutathione peroxidase	↓ in serum of symptomatic than in pre-symptomatic SCA3 carriers (de Assis et al. 2017)	Negative correlation with NESSCA (de Assis et al. 2017)
	Catalase	↑ in serum of SCA3 (Pacheco et al. 2013)	No
Inflammation	Eotaxin	↑ in serum of asymptomatic SCA3 carriers (da Silva et al. 2016)	No
Growth factors	IGFBP1 IGF-1/IGFBP-3 ratio	↑ in serum of SCA3 patients (Saute et al. 2011)	CAG repeat expansion (Saute et al. 2011)
	IGFBP-3 Insulin	↓ in serum of SCA3 patients (Saute et al. 2011)	No
Chaperon	CHIP	↑ in serum and CSF of SCA3 patients (Hu et al. 2019)	SARA and ICARS (Hu et al. 2019)
Metabolism	CYP46A1	↓ in SCA3 cerebellum samples (Nóbrega et al. 2019)	No
	Valine, leucine, tryptophan, and tyrosine	↓ in serum of SCA3 patients (Yang et al. 2019)	No
	Leucine, valine, and tyrosine	↓ in serum of SCA7 patients (Nambo-Venegas et al. 2020)	No
	Ceramides and phosphatidylcholines	↓ in plasma of SCA7 patients (Garali et al. 2018)	No
Enzyme	Sirtuin-1	↓ mRNA in SCA3 patients' fibroblasts (Cunha-Santos et al. 2016)	No
Micro-RNAs	miR-25, miR-125b, miR-29a, and miR-34b	↓ in serum of SCA3 patients (Huang et al. 2014; Shi et al. 2014)	No
	hsa-let-7a-5p, hsa-let7e-5p, hsa-miR-18a-5p, and hsa-miR-30b-5p	Alterations in plasma of SCA7 patients (Borgonio-Cuadra et al. 2019)	Disease onset (Borgonio-Cuadra et al. 2019)

Abbreviations: *CCFS* Composite Cerebellar Functional Score, *CHIP* carboxyl terminus of the Hsp70-interacting protein, *ICARS* International Cooperative Ataxia Rating Scale, *IGFBP* insulin-like growth factor-binding protein, *NfL* neurofilament light chain, *polyQ* polyglutamine, *S100B* protein S 100 B, *SARA* Scale for the Assessment and Rating of Ataxia, *SCA* spinocerebellar ataxia

association between serum NfL and cerebellum and brainstem volumes (Li et al. 2019).

Serum NfL increases already 7.5 years before the expected age at onset for SCA3 carriers (Wilke et al. 2020). NfL levels for SCA3 presymptomatic carriers fall down

between controls and symptomatic carriers levels (Li et al. 2019; Wilke et al. 2020; Peng et al. 2020; Coarelli et al. 2021). SCA7 premanifest carriers with non-cerebellar signs at examination present NfL concentration close or above the cut-off level determined to differentiate controls from carriers (Coarelli et al. 2021). Based on presymptomatic carriers' data (Li et al. 2019; Wilke et al. 2020) and longitudinal data (Coarelli et al. 2021), NfL seems to be a biomarkers that may be used in clinical trials to stratify carriers based on their NfL levels. However, some points remain to be clarified: (i) SCA3 patients present the highest concentration than the other SCAs despite a less severe clinical progression based on SARA score (Coarelli et al. 2021; Jacobi et al. 2015). One possible explanation may be the prominent peripheral nervous system involvement than the other polyQ SCAs; (ii) NfL levels do not change over time in SCAs, similar to ALS, frontotemporal dementia, and atypical parkinsonian syndromes (Gaetani et al. 2019). We may suppose that for these diseases NfL levels reach a plateau that masks the increase due to age. (iii) NfL concentrations for polyQ SCAs fall between the highest levels of ALS or multiple system atrophy and the lowest levels in Friedreich's ataxia or Parkinson disease (Gaetani et al. 2019; Bridel et al. 2019). It may be due to by either different disease progression rates or different levels of peripheral nervous system dysfunction.

2.2 Tau

Another biomarker of neuroaxonal damage is Tau protein that promotes microtubule assembly and stability. This protein is an established marker in Creutzfeldt Jakob disease and Alzheimer's disease (Tumani et al. 2008). In a study including few SCA1, SCA2, and SCA6 patients, Tau levels in CSF were significantly higher in SCA2 carriers than controls (Brouillette et al. 2015). Other proteins were also tested in CSF (α-synuclein, DJ-1, and GFAP) showing a tendency to be higher especially for SCA2 (Brouillette et al. 2015) and indicating the necessity to be reproduced in a larger cohort of patients.

2.3 Astrocytosis and Gliosis

Neuron-specific enolase (NSE) and protein S 100 B (S100B) are markers of neuron damage and gliosis. Serum concentrations of these two proteins are higher in SCA3 patients than controls (Zhou et al. 2011), not tested in other SCA patients. NSE presents a correlations with disease duration and clinical scales (ICARS and SARA), instead of S100B that does not correlate with any clinical parameters (Zhou et al. 2011). In another SCA3 study, only NSE serum level was significantly higher than controls and presented a correlation with depression score (Tort et al. 2005).

2.4 Ataxin-Specific Bioassays

In view of upcoming therapeutic trials that aim to decrease the mutant protein, it seems to be crucial for the development of ataxin-specific assays to monitor the efficacy of these treatments. To date, a time-resolved fluorescence resonance energy transfer (TR-FRET) immunoassay can detect the polyQ-expanded and non-expanded ataxin-3 protein level in blood-derived mononuclear cells from presymptomatic and symptomatic SCA3 carriers (Gonsior et al. 2020). Moreover, polyQ-expanded ataxin-3 protein levels correlated with disease stage and clinical severity assessed by SARA (Gonsior et al. 2020). However, this highly sensitive TR-FRET-based immunoassay cannot measure ataxin-3 level in other fluids such as CSF or plasma and should be validated in other cohorts.

In another study, an electrochemiluminescence immunoassay using the Meso Scale Discovery system detected polyQ-expanded ataxin-3 in CSF and plasma distinguishing controls from SCA3 carriers (Prudencio et al. 2020). In addition, this study showed the strong association between *ATXN3* pathological CAG repeat expansion and the rs7158733 SNP located ~132 nucleotides downstream of the CAG repeat (Prudencio et al. 2020) that could facilitate the allele specific ASO treatment.

Another novel single molecule counting (SMC) ataxin-3 immunoassay is able to measure polyQ-expanded ataxin-3 in plasma and CSF (Hübener-Schmid et al. 2021). Clinical correlations (age at onset and SARA score) are reported with plasma polyQ-expanded ataxin-3 levels. Longitudinal data show that plasma levels remain stable over a 1-year period (Hübener-Schmid et al. 2021).

For the other ataxin proteins, specific bioassays are not yet available.

2.5 Oxidative Stress Biomarkers

Oxidative stress has been implicated in several neurodegenerative disorders. Production of abnormally large amounts of reactive oxygen species was reported for SCA3 (Pacheco et al. 2013). This seems to be caused by a dysregulation of major enzymes implicated in antioxidant capacity: superoxide dismutase and glutathione peroxidase (GPx) activities are lower in symptomatic than in pre-symptomatic carriers (de Assis et al. 2017). On the other hand, catalase activity is increased in the serum of SCA3 patients (Pacheco et al. 2013). The correlation of GPx decrease activity with disease severity suggests that GPx may be a reliable biomarker (de Assis et al. 2017).

In SCA2 presymptomatic and symptomatic carriers, glutathione S-transferases (GST) activity is increased by 21.8% and 5.5%, respectively (Almaguer-Gotay et al. 2014). The role of this enzyme is to protect against oxidative stress and prevent

apoptosis. GST increase activity supports the role of free radical damage in SCAs physiopathology.

2.6 *Inflammation Biomarkers*

Inflammatory genes encoding endopeptidase matrix metalloproteinase 2 (MMP-2) and cytokine stromal cell-derived factor 1α (SDF1α) are upregulated in a cell culture model of SCA3 as well as in human SCA3 pons (Evert et al. 2001). Other proteins involved in inflammation process are significantly increased: amyloid β-protein (Aβ), interleukin-1 receptor antagonist (IL-1ra), interleukin-1β (IL-1β), and interleukin-6 (IL-6) (Evert et al. 2001). Activation of microglia and presence of reactive astrocytes are reported in the brains of SCA3 patients (Evert et al. 2006). Based on these data, a large panel of cytokines has been investigated in a large cohort of presymptomatic and symptomatic SCA3 compared to controls (da Silva et al. 2016). No difference in cytokine levels was detected among the groups except for eotaxin. Higher eotaxin concentrations were observed in asymptomatic carriers than in symptomatic carriers (da Silva et al. 2016). In symptomatic carriers, the level dropped after 1 year (da Silva et al. 2016). One possible explanation may be that the levels of eotaxin released by astrocytes are inversely correlated with disease progression (da Silva et al. 2016).

2.7 *Insulin/Insulin-Like Growth Factor 1 (IGF-1) System*

Abnormalities in the signaling pathway of the insulin/insulin-like growth factor 1 (IGF-1) system (IIS), including IGF-1, IGF binding proteins (IGFBPs), and insulin, are thought to play a role in the physiopathological processes of neurodegenerative diseases as Alzheimer's disease, HD, and polyQ SCAs (Craft and Watson 2004; Cohen and Dillin 2008; Emamian et al. 2003). SCA3 patients show higher serum levels of IGFBP1 and IGF-1/IGFBP-3 ratio than controls (Saute et al. 2011). Inversely, serum levels of IGFBP-3 (that binds more than 80% of peripheral IGF-1 and increases its half-life) and insulin levels are reduced (Saute et al. 2011). β-cell function is preserved in SCA3 patients and the reduction of insulin level is due to an increased peripheral sensitivity to insulin. Higher sensitivity to insulin and lower insulin levels are both related to earlier disease onset (Saute et al. 2011).

IGFBP-1 levels are correlated significantly with CAG repeat expansion (Saute et al. 2011). IGFBP1 may be a biomarker for SCA3 even though its link with expanded ataxin-3 protein remains unclear. One possible explanation could be the endoplasmic reticulum stress induced by mutant ataxin-3 protein that increases IGFBP-1 production in liver (Saute et al. 2011). Even though IGF1 is not significantly higher, it inversely correlates with the volume of medulla oblongata and pons (Saute et al. 2011).

2.8 Co-chaperone Protein

The carboxyl terminus of Hsp-70 interacting protein (CHIP), a co-chaperone protein, is an endogenous binding partner of the mutant ataxin-3. In SCA3 patients, CHIP level is elevated in both serum and CSF, indirectly reflecting mutant ataxin-3 level (Hu et al. 2019). CHIP correlates with disease severity assessed by SARA and ICARS. The main role of CHIP is protein quality control. Ataxin-3 protein directly interacts with CHIP. The affinity between these two proteins increases with CAG expansion causing a cellular homeostasis dysregulation.

3 Biomarkers in Development

3.1 Brain Cholesterol Metabolism

Deregulation of brain cholesterol turnover and metabolism have been associated with several neurodegenerative diseases. 24-hydroxylase (CYP46A1) is the key enzyme of efflux of brain cholesterol, converting the excess cholesterol into 24S-hydroxycholesterol (24OHC) released in systemic circulation (Leoni et al. 2013). Plasma 24OHC is significantly reduced in neurological disorders as Alzheimer's disease, Parkinson's disease, Niemann–Pick disease type C, multiple sclerosis, and HD (Papassotiropoulos et al. 2005; Kölsch et al. 2009; Shobab et al. 2005; Solomon et al. 2009; Leoni et al. 2002). For HD, 24OHC levels decrease with disease progression and striatal volume loss (Leoni et al. 2013). In SCA3 cerebellum samples, CYP46A1 is reduced (Nóbrega et al. 2019). The overexpression by an adeno-associated virus (AAV)-mediated expression of CYP46A1 decreases the ATXN-3 aggregates by activation of autophagy and leads to motor improvement in SCA2 mouse model (Nóbrega et al. 2019). Plasma 24OHC may be a potential biomarker for SCAs as reported for HD, therefore further investigations should be carry on.

3.2 Metabolic Profile

The serum metabolomics profile shows a difference between symptomatic SCA3 patients and presymptomatic carriers or controls (Yang et al. 2019). In SCA3 patients, there is a downregulation of branched-chain amino acids including valine and leucine, and aromatic amino acids as tryptophan and tyrosine (Yang et al. 2019). These metabolites are precursors of some neurotransmitters (serotonin, dopamine, GABA) and have a role in energy metabolism. Fatty acid metabolism is also dysregulated in SCA3 patients with decrease of saturated fatty acid and increase of monounsaturated and polyunsaturated fatty acid fatty (Yang et al. 2019).

Plasma lipidomic analysis in a cohort of polyQ SCAs showed that SCA7 patients differentiate from other polyQ SCAs patients for some ceramides and phosphatidylcholines (Garali et al. 2018). These lipids are strongly expressed in retina and their deficit may be linked to the retinal alterations characteristic for SCA7 rather than other polyQ SCAs.

For SCA7 patients, another study has reported the decreased of branched-chain amino acids, leucine and valine, as well as of tyrosine, with a good sensitivity to discriminate from controls (Nambo-Venegas et al. 2020). Moreover, when regarding only SCA7 carriers, methionine level differentiates early onset from late onset patients (Nambo-Venegas et al. 2020).

3.3 Micro-RNAs

Several studies investigated micro-RNAs (miRNAs) levels in SCAs patients reporting different results. Lower levels of miR-25, miR-125b, miR-29a, and miR-34b are found in serum of SCA3 patients compared to controls (Huang et al. 2014; Shi et al. 2014). Reduced concentrations of miR-9 and miR-181a from CSF derived exosomes of SCA3 patients are reported (Hou et al. 2019). Three miRNAs-mir-9, mir-181a, and mir-494 are decreased in SCA3 human neurons (Carmona et al. 2017). These three miRNAs interact with the ATXN3-3′ UTR downregulating its expression (Carmona et al. 2017). In SCA3 mouse model, the overexpression of these miRNAs reduces the mutant ataxin-3 expression by translation inhibition and mRNA degradation (Carmona et al. 2017). For SCA7, the plasma expressions of four miRNAs (hsa-let-7a-5p, hsa-let7e-5p, hsa-miR-18a-5p, and hsa-miR-30b-5p) differentiate carriers from controls and seem to have a prognostic value discriminating between juvenile and adult onset (Borgonio-Cuadra et al. 2019).

These data could suggest miRNAs as potential biological markers of disease and therapeutic targets. However, their use does not seem to be possible in the short term.

3.4 Sirtuin-1

Sirtuin-1 is a NAD+-dependent deacetylase taking part in several cellular functions as chromatin modulation, cell cycle, apoptosis, and autophagy regulation in response to DNA damage. In SCA3 mice and in SCA3 patients' fibroblasts, sirtuin-1 mRNA levels are lower than controls (Cunha-Santos et al. 2016). In SCA3 mice, the caloric restriction rescues sirtuin-1 with motor improvement (Cunha-Santos et al. 2016). Sirtuin-1 overexpression activates autophagy and increases the mutant protein clearance. This overexpression results in neuropathological changes: activation of autophagy, decrease in neuroinflammation, and reduction in reactive gliosis (Cunha-Santos et al. 2016).

4 Conclusion

This chapter reviews the available biomarkers in blood and CSF for polyQ SCAs. However, the majority of the evidence are reported for SCA3, the most frequent subtype worldwide, and only few longitudinal studies have been conducted with a lower inclusion of presymptomatic carriers. Still many efforts need to obtain an optimal biomarker with diagnostic and prognostic values, reliable to be used in upcoming gene therapy trials. Neurofilaments light chain seems to be currently the best biomarker, already confirmed in several neurological diseases, with a role to monitor drug administration in spinal muscular atrophy (Olsson et al. 2019) and multiple sclerosis (Kuhle et al. 2019). A great interest there is towards the development of ataxin bioassays that are the specific target of ASOs therapy. Other pathways presented in this chapter require validation in larger cohorts.

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Part III
Autosomal Dominant Cerebellar Ataxias

Riluzole in Progressive Cerebellar Ataxias

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Abstract Converging experimental data suggested attempts of using in human beings modulator of ion channels to counteract the neurodegenerative process in progressive cerebellar ataxia (CA). The availability of riluzole, which is already approved for use in clinical practice, with a good safety profile, prompted a repurposing approach.

Two trials supported the attempt to repurpose riluzole in CA. Its symptomatic action (and possibly its slowing effect on disease progression) suggested to consider riluzole in clinical practice as a general front-line therapy, while the diagnostic process is ongoing, as well as an add-on therapy in forms with etiologic treatment.

The research on the possible use of riluzole in CA is currently a hot topic: a precursor of riluzole, troriluzole, is now under scrutiny in two phase 3 trials; in patients with spinocerebellar ataxia type 2 another phase 3 trial is ongoing; a pilot trial is currently in progress in patients with spinocerebellar ataxia type 7.

Keywords Riluzole · Purkinje cells · Deep cerebellar nuclei

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_14

1 Introduction

Cerebellar ataxia (CA) is a disabling syndrome often caused by inherited disorders provoking chronic neurodegeneration. Main symptoms are disturbances of stance and gait, limb ataxia, fine motor disturbances, slurred speech, as well as ocular motor disturbances. Most types of cerebellar ataxia are progressive becoming more and more disabling over time, with important impact on activities of daily living and quality of life of affected patients (Schöls et al. 2004; Diallo et al. 2018). There is no approved medication for the symptomatic or etiologic treatment of degenerative cerebellar ataxia.

Several evidences coming from experimental models and clinical trials converge on a possible effectiveness of riluzole as a symptomatic approach in progressive CA.

The classical neuroprotective action of the drug is ascribed to its interference with the excitotoxic glutamatergic transmission: among other effects, riluzole reduces the size of the readily releasable glutamatergic pool by acting on protein kinase C-dependent Munc18-1 and enhances the astrocyte uptake of ambient glutamate (Lazarevic et al. 2018; Cifra et al. 2013). Other suggested neuroprotective effects include enhancing the expression of BDNF (Katoh-Semba et al. 2002), triggering the glutathione (GSH) synthesis by activating glutamate transporters (Deng et al. 2012), reducing cell death caused by decreasing blood flow in spinal cord injury (Caglar et al. 2018), and preserving glucose metabolism in patients with Alzheimer's disease (Matthews et al. 2021).

An action on potassium channels seems to prevail in CA by the riluzole impact on the firing of the Purkinje cells (PC) and the neurons of the deep cerebellar nuclei (DCN). Loss of inhibition by PC on DCN tonic firing is considered the electrical alteration underlying CA, as demonstrated in a transgenic model that support this mechanism of disease initiation before the occurrence of cerebellar neurodegeneration (Shakkottai et al. 2004). In this study, the openers of small–conductance calcium-activated potassium channels, such as riluzole, may improve DCN hyperexcitability and have therapeutic impact. A work on a rat model of CA (the experimental model of PC neurotoxicity induced by acetyl-pyridine) confirmed that, at least in part, the riluzole neuroprotective effects are mediated by effects on ion channels (Janahmadi et al. 2009). The same group, in a subsequent study through a computational approach, expanded this finding showing that several potassium channels are involved in the beneficial effects of riluzole in CA (Abbasi et al. 2013). In this context, riluzole enhances the recently described TWIK-related potassium channel-1 (TREK-1), and the intracellular expression of heat shock proteins, which have a neuroprotective role and counteract the blood–brain barrier dysfunction (a trigger of the inflammatory component underlying several neurodegenerative diseases) (Bittner et al. 2013).

Concerning experimental models of diseases from polyglutamine expansion (the causative genetic changes of most inherited dominant CA), a recent work reported on a transgenic spinocerebellar ataxia type 2 (SCA2) mouse. It added evidence to the fact that a modulator of calcium-activated potassium channels is a therapeutic

target for CA, being a potential beneficial agent for SCA2 and possibly other SCAs. Using cerebellar slices, the authors showed that an oral delivery of a more selective positive modulator of SK2/3 channels (NS13001) alleviated behavioral and neuropathological phenotypes of aging SCA2 transgenic mice (Kasumu et al. 2012).

Along the same line, a model based on induced pluripotent stem cells (iPSCs) and self-organizing culture technologies contributed to disentangle the pathogenic pathways of SCA6 GAG repeat, and confirmed the beneficial effect of riluzole on antagonizing the degeneration of PC in this condition; in particular, riluzole, as well as other compounds such as thyrotropin-releasing hormone (TRH), turned out to be effective in reverting the vulnerability of iPSC-derived Purkinje cells induced by nutrient depletion (Ishida et al. 2016).

Overall, experimental data definitely warrant attempts in human beings with modulator of ion channels in progressive CA. The availability of a drug, such as riluzole, that is already approved for use in clinical practice, with a good safety profile, and with a plausibly multiple neuroprotective actions prompts a repurposing approach that is currently in progress.

2 Symptomatic Effects of Riluzole in Progressive Ataxias

The first trial studying the symptomatic effects of riluzole in CA was reported in 2010 (Ristori et al. 2010) on 40 patients with various forms of disease (inherited CA, neurodegenerative conditions, immune-mediated forms, and cases of unknown origin). Twenty patients were randomly assigned to receive riluzole and twenty to receive placebo. CA was assessed by the International Cooperative Ataxia Rating Scale (ICARS), a 100-point semi-quantitative scale that provides a total score and subscores: increased or decreased scores indicate respectively worsening or improvement in CA (Trouillas et al. 1997). Notwithstanding the heterogeneous nature of each study arm, no significant difference was found between the two groups in the main baseline characteristics (mean age, male/female ratio, disease duration, type of ataxic syndrome, and ICARS total scores and subscores). The outcome measures were the proportion of patients with a decrease in at least 5 points in the ICARS total score after 4 and 8 weeks compared with the baseline score, as well as the mean changes from the baseline to post-treatment ICARS (total score and subscores at 8 weeks).

The difference between riluzole and placebo groups in the primary outcome was already evident after 4 weeks (9/19 [47.4%] vs. 1/19 [5.3%]; odds ratio [OR] = 16.2; 95% confidence interval [CI]: 1.8–147.1) and became clear cut after 8 weeks (13/19 [68.4%] vs. 1/19 [5.3%]; OR = 39.0; 95% CI: 4.2–364.2). Concerning the mean change in the ICARS scores from baseline after 8 weeks of treatment, we observed an improvement in the riluzole group in both the total score (−7.05 [4.96] vs. 0.16 [2.65]; $p < 0.001$) and three subscores: −2.11 [2.75] vs. 0.68 [1.94] for static function, −4.11 [2.96] vs. 0.37 [2.0] for kinetic function, and −0.74 [0.81] vs. 0.05 [0.4] for dysarthria; $p < 0.001$ for each.

In this pilot study, mild adverse events were observed in three patients treated with riluzole: in two cases an increase in alanine aminotransferase (<1.5 times above normal limit), and a transient vertigo in the third case, which are within the known safety profile of the drug.

The second trial was reserved to hereditary CA and was designed to obtain results over a 1-year period to assess the stability of the riluzole effects in these forms of disease (Romano et al. 2015). The analysis was performed on 28 treated and 27 placebo patients with SCA or Friedreich ataxia. The outcome measures were based on the Scale for the Assessment and Rating of Ataxia (SARA), a reliable and valid measure of ataxia, widely considered an appropriate end point for clinical trials (Schmitz-Hübsch et al. 2006). The analyses were done at month 3 and 12 of the study period.

The demographic and baseline characteristics of the two groups did not differ. The proportion of patients with a decreased SARA score after 12 months was significantly higher in the riluzole than the placebo arm: OR = 8.00, 95% CI: 1.95–32.8, $p = 0.002$. The mean changes of SARA score compared to baseline were significantly different between the two groups at both 3 and 12 months. We found negative mean changes for treated patients and positive mean values for placebo group: -1.00 ± 1.75 vs. 0.50 ± 2.28 and -1.02 ± 2.15 vs. 1.67 ± 2.63. The mean difference in SARA score between the two groups was −1.5 (95% CI: −2.59 −0.40) at 3 months, and − 2.68 (95% CI: −3.98−1.39) at 12 months. The worsening of cerebellar ataxia was more frequent in the placebo than in the riluzole arm: 10/27 (37%) vs. 4/28 (14%) patients at 3 months; 13/27 (48%) vs. 4/28 (14%) patients at 12 months (OR = 5.39, 95% CI: 1.51–22·54, $p = 0.006$). To evaluate the clinical relevance of riluzole effects, we performed a post-hoc analysis, considering the proportion of patients reaching the SARA score of 5.5 or lower (indicative of mild dependency in the performance of daily living) (Kim et al. 2011) at 12 months: we found a significant difference between treated and placebo group, following adjustment for baseline characteristics (OR = 5.87, 95% CI: 1.07–32.35, $p = 0.04$). Also, in this longer trial, no severe adverse events occurred. No problem of treatment compliance was recorded. The sporadic, mild adverse events were, once again, within the known safety profile of the drug. These promising results have been challenged by a recent multicenter, randomized, double-blind, placebo-controlled trial in a subtype of CA (moderately affected patients with SCA2), where the authors failed to show significant differences between riluzole and placebo (Coarelli et al. 2022). Overall, the previous trials support the attempt to repurpose riluzole that is currently indicated in amyotrophic lateral sclerosis, in CA: its symptomatic action, and possibly its effect in slowing disease progression after 1 year, suggest to consider riluzole in clinical practice. It may be useful as a general front-line therapy, while the diagnostic process is ongoing. In fact, the identification of CA causes is often time-consuming, and having at disposal a drug capable of improving or stabilizing the patient's conditions may result very useful. Moreover, riluzole may turn out to be effective even as add-on therapy in the forms of cerebellar ataxia where specific agents are known to counteract the disease progression. In a recent work on the cerebrotendinous xanthomatosis, which has a standard therapy with chenodeoxycholic acid, riluzole was used as an add-on therapy for CA, since neurological

improvement is not typically seen after such standard treatment. The authors describe in this case a quantifiable improvement on SARA score after the addition of riluzole, which supports its usefulness as a symptomatic approach in several forms of progressive CA (Weissfeld and Ratliff 2018).

3 Future Perspectives

The research on the possible use of riluzole in CA is currently a hot topic. A precursor of riluzole, troriluzole, sponsored by the Biohaven Pharmaceuticals, Inc., with similar pharmacologic and better pharmacokinetic properties, is now under scrutiny. Specifically, it reduces the synaptic levels of glutamate by augmenting the expression and function of the glial glutamate transporters (i.e., EAAT2) responsible for glutamate synaptic clearance (Grassi et al. 2021). Two parallel-group, randomized, placebo-controlled phase 3 trials on the efficacy of troriluzole (NCT03701399 and NCT02960893) are ongoing (one in 210 adults with a known or suspected diagnosis of specific forms of SCA treated for 48 weeks; one in 141 adults, treated for 8 weeks). A randomized, double-blind, placebo-controlled pilot trial, with a lead in phase, is currently in progress in patients with spinocerebellar ataxia type 7 (NCT03660917). It stems from preliminary data obtained by two groups (one in Italy and the other in Florida, USA) on an off-label use of riluzole in three cases of SCA7 (an autosomal dominant form of CA with important retinopathy). Improvement of CA at the SARA score was reported in all patients, along with improvement and stability of visual acuity in two of them (the third one had an overt visual loss when riluzole was started).

In summary, the repurposing approach of riluzole in CA seems to hold promises, though it has not yet reached a definitive demonstration of efficacy that warrants indication in clinical practice. Currently an off-label use is sporadically implemented in various forms of CA, exploiting the reported trials. The trajectory speed of a repurposed drug in rare diseases is not necessarily fast (Fig. 1). However, the availability of riluzole in the therapeutic armamentarium of neurologists dealing

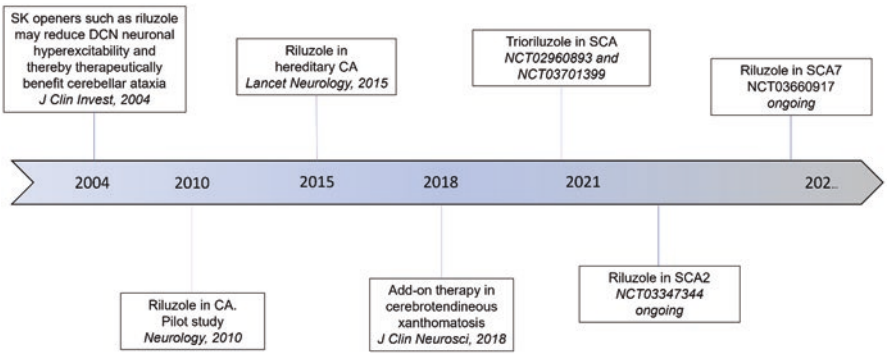

Fig. 1 Riluzole story in progressive CA

with CA patients seems plausibly not so far, even as an add-on therapy of possibly coming etiologic treatments. As strengthened by the recent finding of Coarelli et al., multicenter trials with an informative sample size in specific subgroups/subtypes of CA may help to design informative studies to reach the possible definitive demonstration of efficacy and the potential riluzole use in clinical practice.

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ASOs Against ATXN2 in Preclinical and Phase 1 Trials

Stefan M. Pulst

Abstract Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominantly inherited neurodegenerative disease caused by DNA CAG repeat expansion. The mutation is in the coding 1st exon of the *ATXN2* gene and results in an expanded polyglutamine (polyQ) domain. SCA2 is characterized by progressive ataxia and involves primarily Purkinje cells (PCs) but also other neurological systems. Some individuals with ATXN2 mutations can present as pure Parkinson or Lou Gehrig disease. Long normal ATXN2 alleles are risk alleles for amyotrophic lateral sclerosis. Comparison of mouse models expressing mutant ATXN2 (Pcp-tg-hATXN2-Q127; BAC-hATXN2-Q72) and Atxn2−/− mice clearly favors a predominant gain-of-function mechanism of repeat-expanded ATXN2 based on morphologic, transcriptomic, and slice physiology analyses. The lack of a neurodegenerative phenotype in Atxn2−/− mice led us to adopt a strategy of targeting wild-type and mutant ATXN2 with antisense oligonucleotides (ASOs). In two transgenic models, we were able to provide proof-of-principle data that targeting ATXN2 with intracerebroventricular injection of ASOs can slow progression of motor dysfunction. ASO treatment also improved expression levels of PC-specific proteins and PC firing frequencies in the acute cerebellar slice. An ASO targeting ATXN2 is currently in phase 1 human trials (BIIB105).

Keywords Ataxia · ATXN2 · Antisense oligonucleotide · Mouse

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_15

1 SCA2 Clinical Characteristics

What is now known as SCA2 was initially described in a number of pedigrees characterized by cerebellar ataxia and slow eye movements in India (Wadia & Swami 1971). Nearly two decades later, a large population with ataxia and ophthalmoplegia was described in a founder population in the province of Holguin in eastern Cuba (Orozco Diaz et al. 1990). In 1993, the SCA2 locus was mapped to human chromosome 12q24 in several pedigrees, including Cuban individuals (Gispert et al. 1993). In the same year, we mapped an American-Italian pedigree with significant anticipation of disease onset to this region (Pulst et al. 1993).

The causative mutation was identified in 1996 as a DNA repeat expansion mutation by three groups (Pulst et al. 1996; Imbert et al. 1996; Sanpei et al. 1996). Most commonly, the *ATXN2* gene has 22 CAG repeats in controls, while ≥33 CAG repeats cause SCA2 (Fernandez et al. 2000). SCA2 is characterized by anticipation with strong inverse correlation between age of onset and CAG repeat length and meiotic instability (Figueroa et al. 2017). In the Cuban founder population, variance in age of disease onset is determined by CAG repeat size, genetic modifiers, and stochastic factors in a proportion of 50%, 25%, and 25% (Figueroa et al. 2017).

The initial clinical characterizations showed that SCA2 was a neurodegenerative disease that predominantly affected the cerebellum. While patients with SCA2 have many of the clinical characteristics that define the SCAs as a group of neurodegenerative disorders, the SCA2 phenotype, when assessed across a large number of individuals, can be clinically distinct. Gait ataxia, considered a characteristic of SCA, is the most noticeable symptom and is often the presenting symptom and sign (Pulst et al. 1993; Luo et al. 2017). A distinguishing feature of SCA2, not necessarily by its presence, but by its severity, is the slowing of saccadic eye movements (Geschwind et al. 1997; Ashizawa et al. 2013; Gwinn-Hardy et al. 2000).

Although gait ataxia is usually the first symptom, onset may also coincide with muscle cramping. Gait ataxia is followed by multiple other symptoms characteristic of cerebellar dysfunction such as appendicular ataxia with instability of stance, dysarthria, and ocular signs. In retrospect, it has become clear that some, but not all, of the families with slow eye movements described by Wadia in India had mutations in the *ATXN2* gene (Wadia and Swami 1971; Wadia et al. 1998).

Some SCA2 patients may show prominent involvement of basal ganglia or upper and lower motor neurons. This led to subsequent identification of individuals with pure outlier phenotypes. Gwinn-Hardy and colleagues described Taiwanese patients with tremor-predominant L-DOPA responsive Parkinson disease that were found to have ATXN2 mutations with later confirmation in other ethnic populations (Gwinn-Hardy et al. 2000; Payami et al. 2003). A pure ALS phenotype can also occur with *ATXN2* mutations (Tazen et al. 2013; Neuenschwander et al. 2014).

ATXN2 repeat expansions can act as recessive, dominant, or risk alleles depending on CAG repeat size (reviewed in Pulst 2018). Alleles with ≥33 repeats are dominant in causing adult-onset ataxia, and alleles of 31 and 32 repeats are recessive. Long normal repeats in *ATXN2* are risk alleles for ALS (Elden et al. 2010; Tazen et al. 2013; Neuenschwander et al. 2014).

1.1 SCA2 Models

ATXN2 is highly conserved in evolution. Its yeast ortholog is poly(A)-binding protein-1 (Pab1)-binding protein (Pbp1). In *C. elegans*, Kiehl and colleagues found that Atx2 played an essential role in patterning using RNAi (Kiehl et al. 2000). In the fly, Satterfield and Pallanck (2006) showed that dAtx2 assembled with polyribosomes and poly (A)-binding protein (PABP). Physical interaction with PABP was mediated by the N-terminal Lsm/Lsm-associated domain (LsmAD) and the PAM2 motif in dAtx2.

In a screen for modifiers of SCA3 neurodegeneration, the Bonini group pointed to an important function of fly atx2 in neurodegeneration. They showed that normal activity of Atx2 was critical for SCA3 degeneration, depending in particular on the PAM2 motif (Lessing and Bonini 2008). Similarly, dAtx2 mediates mutant Atx1 neurodegeneration in the fly (Al-Ramahi et al. 2007).

Mouse and human ATXN2 are highly homologous (Nechiporuk et al. 1998). At the nucleotide level, identity was 91% and at the amino acid level 89%. The region flanking the glutamine is significantly less conserved than the rest of the protein. Lack of conservation could potentially argue for the generation of mouse models using human cDNAs or human BACs as compared with knock-in models.

We and others have produced multiple SCA2 mouse models, including transgenic and knockout models. Recent reviews describe these mouse lines in detail (Alves-Cruzeiro et al. 2016; Scoles and Pulst 2018; Cendelin et al. 2022). In the following paragraphs, we will focus on those models that directly led to the development of ASOs for SCA2 and ALS and not discuss other transgenic models (Aguiar et al. 2006) or models using knock-in strategies of mutant ATXN2 CAG repeats (Arsović et al. 2020).

1.2 Pcp2-ATXN2 *Transgenic Mice*

We generated two transgenic lines expressing ATXN2 with mutant repeats of Q58 and Q127 under the control of the Purkinje cell protein 2 (*Pcp2*)/*L7* promoter (Huynh et al. 2000; Hansen et al. 2013). In the Pcp2-ATXN2[Q58] mice, rotarod testing demonstrated an ATXN2 dose-dependent motor phenotype for *ATXN2*-Q58 mice first observed at 6 months of age. PCs contained cytoplasmic but not nuclear inclusion bodies. The ATXN2-Q58 mouse was also used in studies demonstrating that SK positive modulators restored ATXN2 mouse motor and electrophysiological phenotypes (Liu et al. 2009; Kasumu et al. 2012; El-Sayed et al. 2022). Similar studies were performed using these mice with chlorzoxazone (Egorova et al. 2021).

To enhance the motor phenotype compared with the ATXN2-Q58 mouse, we generated a line expressing the entire human cDNA with 127 repeats under control of the Pcp2-promoter. This Pcp2-*ATXN2*-Q127 line had an earlier motor onset at 8 weeks as well as presence of cytoplasmic inclusions (Fig. 1). This line was studied

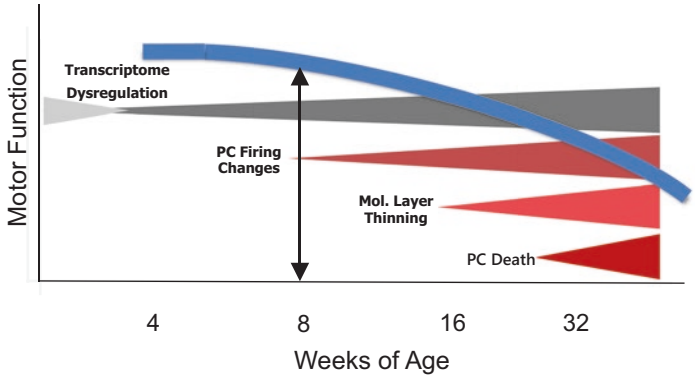

Fig. 1 Schematic of the time course of different aspects of the phenotype in Pcp2-ATXN2[Q127] mice. The blue line denotes the motor performance on the accelerating rotarod, which becomes abnormal at 8 weeks. Transcriptomes show 2 sets of differentially expressed genes (DEGs), the DEGs shown in light grey representing a developmental set, the DEGs in dark grey representing a set of progressively dysregulated mRNAs mirroring neurodegeneration

in greater detail than the Q58 line using molecular, motor, and electrophysiology in the acute cerebellar slice (Hansen et al. 2013; Pflieger et al. 2017). Although subtle mRNA expression changes were detected at 4 weeks in cerebellar mRNAs, more significant changes were seen at onset of the motor phenotype at 8 weeks. A genome-wide transcriptomic study comparing cerebellar RNAs of wild type and Q127 mice at 1 day, 3 and 6 weeks of age confirmed these results (Pflieger et al. 2017). The number of differentially expressed genes (DEGs) with stringent cutoff criteria increased from 138 at day 1 to 458 at 3 weeks and 434 at 6 weeks. Only 3 DEGs were shared across all 3 time points, whereas 87 DEGs were in common at 3 and 6 weeks (Pflieger et al. 2017). For top cerebellar DEGs, we queried the literature to identify commercially available antibodies to verify reduction in protein levels. These included RGS8, FAM107B, CEP76, HOMER3, as well as PCP2 and PCP4 (Scoles et al. 2017). On the other hand, levels of phosphorylated mTOR and p62 are increased (Paul et al. 2018, 2021).

PCs fire spontaneously at high rates, and absent synaptic inputs, firing is regular (reviewed in Meera et al. 2016). Altered PC intrinsic firing has been observed in several mouse models of ataxia including SCA2 (reviewed in Cook et al. 2021). Using the acute cerebellar slice, we examined the intrinsic spontaneous firing of PCs in wild type and Pcp2-ATXN2[Q127] mice at various time points (Hansen et al. 2013). The extracellular recordings were made by placing an electrode adjacent to a PC body, a technique, which permitted sampling of dozens of PCs in a single slice (Meera et al. 2017). We found that PCs reached their adult firing frequency of about 40 Hz at 6 weeks of age, which was maintained in wild-type mice through 40 weeks of age, but progressively decreased in mutant mice. Of note, the PC physiology in the acute cerebellar slice may differ from that observed in vivo using ATXN2-58Q mice (Egorova et al. 2021).

A significant reduction in PC spontaneous firing was first seen at 8 weeks, a time point that marked the onset of the motor phenotype on the accelerating rotarod. In summary, these results confirmed that the mouse model replicated salient feature of human SCA2, that is, an adult-onset neurodegenerative disease with only very minor developmental components.

1.3 SCA2 BAC Transgenic Mice

Testing of RNA-based therapies ideally requires mouse models that result in expression of the entire human heterogeneous nuclear RNA under control of the endogenous promoter. To address the potential problems of cDNA-based transgenes, we aimed at generating a mouse model that expressed the entire human *ATXN2* gene in as a bacterial artificial chromosome (BAC).

The human *ATXN2* gene consists of 25 exons and spans a total of 147 megabase pairs (Nechiporuk et al. 1997; Sahba et al. 1998). The largest *ATXN2* transcript is 4699 bp long including a 162 bp 5′-UTR and a 601 bp 3′-UTR. There are two in-frame start codons, the second one located just 12 bp upstream of the CAG repeat. The predicted molecular weight for ATXN2 is 144 kDa, when translated from the first start codon, and smaller by 17 kDa, when translated from the second one (Scoles et al. 2012).

We introduced a BAC containing the entire 176 kb *ATXN2* gene region including 16 kb upstream sequence and 2.5 kb downstream sequence into the mouse germline. Presently, we have two SCA2 BAC lines expressing ATXN2-Q22 or ATXN2-Q72 (Dansithong et al. 2015). Although the Q22 line has no motor, transcriptomic or neurophysiological phenotype, it has played a crucial role in the development of gene-targeted therapies as this line breeds very well and permits easy determination of target engagement of wild-type *ATXN2* mRNA.

The Q72 line has an onset of a rotarod phenotype at 8 weeks. To cross-validate our different animal models, we examined transcriptomes and found that there was a significant overlap with DEGs seen in the Q127 line as a result of BAC expression in PCs (Dansithong et al. 2015). Both models also share changes in intrinsic PC excitability (Hansen et al. 2013; Scoles et al. 2017); changes in BAC-Q72 are shown in Fig. 2.

2 ASO Development Targeting Wild-Type and mt ATXN2

2.1 Atxn2 *Knockout Mice*

A major piece of evidence needed prior to developing therapies based on targeting ATXN2 levels directly, and especially targeting wild type (wt) and mutant (mt) alleles simultaneously, is knowledge regarding the effects of ATXN2 knockout

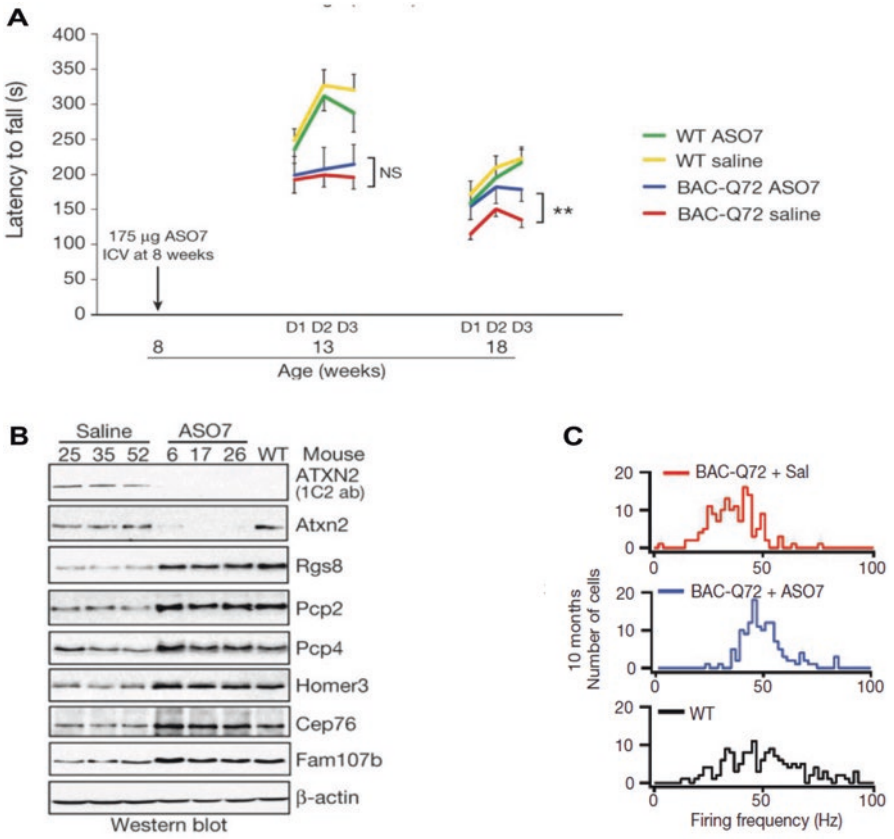

Fig. 2 Effects of ASO treatment on multiple phenotypes in the BAC-ATXN2[Q72] mice. (**a**) Improved rotarod performance of BAC-Q72 mice treated at symptom onset at 8 weeks. (**b**) Near normalization of key PC proteins by ASO treatment. Note significant reduction in mutant human ATXN2 as recognized by the 1C2 antibody, which targets expanded polyQ domains. (**c**) Analysis of intrinsic PC firing in the acute cerebellar slice. ASO7 restores normal firing frequency in BAC-Q72 mice. (Modified from Scoles et al. (2017))

in vivo. Specifically, it was not known, whether CAG repeat expansions in the *ATXN2* gene acted exclusively as a dominant gain-of-function allele or also had a loss-of-function component. To answer these questions, we generated *Atxn2* deficient mice for comparison with transgenic mice (Kiehl et al. 2006; Huynh et al. 2009).

Null mice were viable and did not have obvious morphologic central nervous system (CNS) deficits. Litters, however, showed significant sex-specific segregation distortion. Null and, to a lesser degree, heterozygote mice developed adult-onset hyperphagia and obesity. There was hyperactivity in the open cage and lack of cued and contextual fear conditioning. Long-term potentiation was impaired in the amygdala, but not in hippocampus. This was consistent with a lack of a phenotype in the Morris water maze in contrast with the observed lack of fear conditioning

(Huynh et al. 2009). Similar results were seen in an independently generated knockout mouse (Lastres-Becker et al. 2008).

In contrast to transgenic lines, we did not detect changes in intrinsic PC firing or significant transcriptomic changes (Pflieger et al. 2017). Lack of significant molecular, physiologic, or morphologic changes in the cerebellum of *Atxn2*$^{-/-}$ mice supported our notion that mutant ATXN2 acts predominantly by gain-of-function and led to our efforts targeting both wild-type and mutant *ATXN2* alleles using antisense oligonucleotides (ASOs).

2.2 Establishing Cerebellar RNA and Protein Markers for Preclinical Studies

Staining of PCs and their dendrites with antibodies to the calcium-binding protein calbindin 28K (CALB1) has been a staple for characterizing cerebellar degeneration, often with a focus on lobule VI as it can be easily identified. CALB1 is a protein that is highly expressed in PCs and involved in intracellular calcium regulation. In addition to intensity, CALB1 staining can be conveniently used to measure thickness of the molecular layer. The procedure generally involves determining a region of interest on a tissue slide and then measuring intensity with a program such as ImageJ.

With an eye on developing therapeutics, we aimed to develop methods that would sample entire cerebellar hemispheres in an operator-independent way. We began by adding quantitative reverse transcribed polymerase chain reaction (qPCR) of *Calb1* mRNA to our evaluation panel. This approach proved to be highly reproducible and allowed us to examine levels at different time points along the disease process. Levels of *Calb1* were normal at birth, significantly downregulated as early as 4 weeks of age, and exhibited a continual decline of expression after that (Hansen et al. 2013).

Our analytic methods progressing from semi-quantitative immunohistochemistry to quantitative PCR of key PC-specific genes led to the use of genome-wide transcriptional profiling. The identification of top dysregulated genes (DEGs) subsequently led to the development of protein assays that allowed us to follow progression of disease and response to therapeutic interventions (Pflieger et al. 2017; Scoles et al. 2017, 2020).

2.3 RNA-Based SCA2 Therapeutics

As transgenic models suggested a dominant (toxic) gain-of-function mechanism for pathogenesis with further support from observations in *Atxn2* null mice, we began investigating strategies to reduce ATXN2 expression. We were guided in our approach by recognition that ATXN2 disease phenotypes affect multiple neuronal

systems and that a successful treatment would have to be able to reach neurons (and potentially glia) not only in the cerebellum, but also in brainstem, cerebral hemispheres, and spinal cord. In two independent small compound screens we identified several compounds that reduced ATXN2 expression in vitro, among them cardiac glycosides, but these were predicted to be highly toxic and to have low capacity to cross the blood-brain barrier (Scoles et al. 2022).

With development of ASOs for neurologic diseases (reviewed in Scoles et al. 2019), we explored the feasibility of targeting ATXN2 in vitro and in vivo using phosphothiorated gapmer ASO in collaboration with Ionis Pharmaceuticals in 2012 (Carlsbad, USA). Gapmer ASOs have different modifications at their ends compared with the middle of the molecule. The ASOs were 20 bp in length and the backbone was phosphorothioate throughout. The terminal 5 bps at each end of the oligonucleotide had a 2′-*O*-methoxyethyl group (MOE) (Rigo et al. 2014; Scoles et al. 2017). The modifications are predicted to reduce degradation of the ASO by nucleases and at the same time to increase specificity of target mRNA interaction and degradation by RNase-H (Bennett and Swayze 2010; Crooke et al. 2021).

To identify potent ASOs, we conducted an in vitro screen with ASOs designed in silico for targeting human *ATXN2*. A total of 152 ASOs were tested in human HepG2 cells at a concentration of 4.5 μM. Delivery occurred by electroporation in two 384-well plates and *ATXN2* expression evaluated by qPCR. The 7 best ASOs were progressed to in vivo testing.

For in vivo testing, 250 μg of the 7 best ASOs were injected into the right lateral ventricle of wild type and BAC-Q72 mice and ATXN2 reduction determined after sacrifice at 7 days. To assess astroglial and microglial activation we measured cerebellar *Gfap* and *Aif1* expression by PCR. Although ASO7 reduced mouse *Atxn2* the most by 50% in wild-type mice, none of the changes of ASO7 or other ASOs were significant, which was not surprising as ASOs were directed against human *ATXN2*. One ASO significantly elevated *Aif1* expression. Three ASOs including ASO7 reduced human ATXN2 significantly in BAC-Q72 mice compared with saline injected mice. These 3 ASOs were also confirmed in their ability to reduce ATXN2 in ATXN2-Q127 mice.

When the best ASOs were analyzed at 10 weeks after injection, only ASO7 was without glial activation and we focused detailed analysis on this ASO, which targets *ATXN2* exon 11. Its uptake into PCs was confirmed by using a proprietary antibody recognizing the ASO backbone developed by Ionis Pharmaceuticals (Carlsbad, USA).

Mice were treated with ASO7 at an early symptomatic stage (8 weeks of age) or saline. Behavior was tested on the accelerating rotarod. The behavior paradigm involved testing three times per day on 3 consecutive days with the rod accelerating from 0 RPM by 1 RPM every 9 seconds. All mice including wild-type mice had fallen off the rod at 7.5 min (50 RPM). We chose this paradigm with relatively rapid acceleration and RPMs > 40 to avoid a ceiling effect that is observed in wild-type mice, which often reach the end in other rotarod paradigms without falling. This can potentially lead to a falsely reduced standard deviation in wild-type mice.

We conducted these preclinical trials following recommendations outlined by Landis and coworkers (Landis et al. 2012). This included strict randomization and

blinded evaluation with replication in two different animal models. We also opted for the use of B6/D2 hybrid mice to introduce a measure of genetic diversity. All mouse breeding followed recommendations by the Jackson laboratories. For statistical analyses of rotarod data we used Generalized Estimating Equations with the independent correlation option in Stata 12. The independent correlation option was employed, because regressions for wild-type mice frequently have more positive correlation coefficients than SCA2 mice in the 3-day rotarod paradigm.

We tested ASO7 in the Pcp2-Q127 and the BAC-Q72 models with a single intracerebroventricular (ICV) injection of 210 and 175 μg, respectively, at 8 weeks of age, when these lines are beginning to show motor deficits. Control groups were injected with saline. Our primary outcome criterion was improved motor performance, secondary outcome was reduction of ATXN2 at the end of the experiment, and tertiary outcomes were restoration of PC-specific key proteins and PC intrinsic activity.

In both models, saline-treated animals displayed the previously described deterioration of motor performance, whereas ASO-treated mice stabilized in their performance and had significantly improved performance compared with saline-treated animals. In the experiment using BAC-Q72 mice with ASO and saline treatment of wild-type and mutant mice, 10 weeks after ICV injection ASO7 had no adverse effects in wild-type mice and improved motor performance in BAC-Q72 to the level of wild-type mice (Fig. 2a).

At the endpoint (19 or 22 weeks of age), we determined the cerebellar expression of ATXN2 as a secondary endpoint. We found that ASO7 had shown long-lasting target engagement at the level of *ATXN2* mRNA and ATXN2 protein and reduced both by ≥75% in the two models.

To further demonstrate a direct effect on PC survival and function we employed quantitative PCR and western blot analyses of cerebellar extracts as well as assessment of intrinsic PC activity in the cerebellar slice. Our previously established key PC marker mRNAs and proteins (Cep76, Fam107b, Homer3, Rgs8, Pcp2, and Pcp4) showed near normalization of mRNA and protein expression. Subsets of mice were tested to determine the effect of the ASO7 on PC physiology in the acute cerebellar slice. Treatment with ASO7 treatment restored the mean PC firing frequency to that observed in age- matched wild-type mice.

3 ALS and ATXN2 ASO Phase 1 Study

In parallel with our studies in SCA2 models, the Gitler laboratory explored the role of Atxn2 in ALS with regard to TDP43 toxicity (Elden et al. 2010; Becker et al. 2017). The Gitler lab had employed yeast-2-hybrid screens to identify TDP43 as an ATXN2 interactors followed by showing that knockdown of dAtx2 improved a TDP43 induced phenotypes in the fly (Elden et al. 2010). They had also examined human genetic evidence by comparing different classes of ATXN2 repeat alleles and their association with human ALS. They found that presence of alleles with ≥27

repeats represented significant genetic risk factors for ALS (Elden et al. 2010). We and others subsequently narrowed the risk alleles to ≥30 repeats with progressively increasing risk and reaching >10-fold for individuals with ATXN2^{Q32} (reviewed in Neuenschwander et al. 2014). Frequency of the 27-repeat allele is highly variable in different populations and unrecognized population stratification can result in erroneous risk assessments. It is now well accepted that alleles from 27 to 29 repeats do not confer increased risk for ALS (Fig. 3). The molecular basis for the steep increase in ALS risk observed with the addition of a single glutamine (or CAG/CAA repeat) is currently not known.

Additional support for a role of ATXN2 in ALS has come from analysis of spinal cord transcriptomes in BAC-Q72 mice (Scoles et al. 2020). Scoles and colleagues identified DEGs in the innate immunity, the complement system, and lysosome/phagosome pathways and showed partial reversal of DEGs after ASO7 treatment. Of note, many DEGs and pathways overlapped with transcriptomes in other ALS mouse models and in those obtained by analysis of human postmortem ALS spinal cords.

TDP43 is a protein directly mutated in ALS patients and >90% of ALS patients have TDP43 aggregates in spinal neurons. Gitler and colleagues therefore analyzed whether *Atxn2* knockdown by genetic interaction or Atxn2 ASOs improved TDP43 toxicity in a mouse model overexpressing wild-type TDP43 (Becker et al. 2017). Both genetic interactions using ATXN2 knockout mice and *Atxn2*-ASO treatment by ICV injection at postnatal day 1 had a marked effect on survival in this model with some long-term survivors seen. Of note, median survival and percentage of long-term survivors increased with reducing Atxn2 abundance with the best effects seen with complete absence of Atxn2.

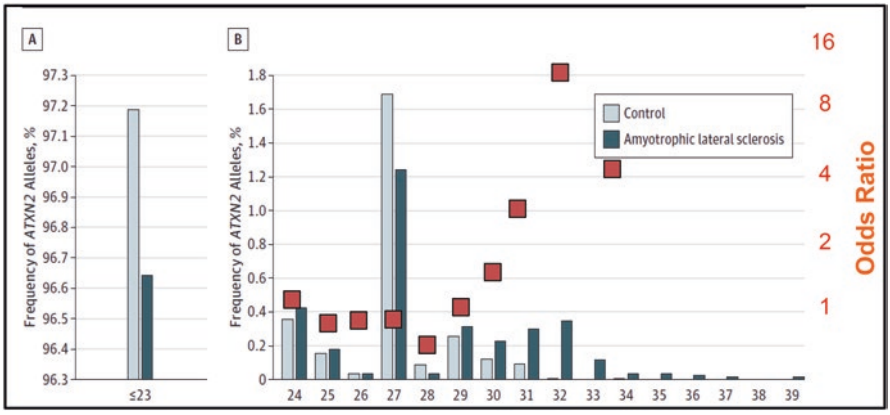

Fig. 3 Meta-analysis of the distribution of *ATXN2* repeat alleles in control and ALS populations. (**a**) Frequencies of the most common alleles with 22 and 23 repeats (rare alleles <22 are included). (**b**) Frequency of rare longer "normal" alleles. Risk of ALS does not increase until 30 repeats, but then increases steeply. Orange squares indicate odds ratio for developing ALS. (Modified from Neuenschwander et al. (2014))

3.1 Phase 1 Clinical Trial (BIIB105)

The encouraging preclinical proof-of-concept studies led to further development of improved ASOs to ATXN2 by Ionis Pharmaceuticals in collaboration with our group at the University of Utah. An IND for an ATXN2 ASO (Ionis 541) was filed on March 31, 2020 and a phase 1 dose escalation study supported by Biogen began on Sept 1, 2020, designated BIIB105. The study sponsor made the interesting decision to target wild-type ATXN2 in ALS patients, a population that does not carry mendelian deterministic *ATXN2* alleles, rather than mutant expanded *ATXN2* in SCA2 patients.

BIIB105 is in the process of recruiting two different ALS populations. The first consists of ALS patients without family history and without mutations in the *SOD1* or *FUS* genes that will receive increasing single doses of the Ionis541 ATXN2 ASO. It is interesting to note that the study sponsors did not opt to test for repeat expansions in the *C9ORF72* gene in this context, or for that matter, employed whole exome sequencing (WES) to look for other established ALS genes.

A second cohort is examining dose escalation in individuals with ALS and at least one ATXN2 allele with 30–33 CAG/CAA repeats. As of January 31, 2022, BIIB105 is ongoing and recruiting patients. It is hoped that successful dose finding in BIIB105 will lead to phase 1/2 trials in SCA2 patients.

4 Conclusions and Outlook

It took almost 50 years after the first description of SCA2 and 25 years after gene discovery to bring RNA-based therapies targeting ATXN2 into clinical trials. In 1996, only few could have imagined that ATXN2 alleles could act in a recessive and dominant fashion and also as risk alleles for other adult-onset neurodegenerative diseases. Animal models using expression of transgenes and Atxn2 knockout have been instrumental in understanding pathogenesis and were the basis for proof-of-principle studies in therapy development. ATXN2 is now being explored as valid target for the development of biologicals for SCA2 and other neurodegenerative diseases. ASO-based therapies may only represent the beginning and will likely be followed by viral and small molecule approaches.

Acknowledgments I want to thank SCA2 patients, their spouses and partners, who often serve as caregivers, on several continents for their help with understanding SCA2. Special gratitude and recognition go to members of my laboratory over the last three decades for their intellectual and technical contributions.

FundingThis work was supported by grants R01NS33123, R56NS33123, RC4NS073009, UO1 NS103883, and R37NS033123 from the National Institutes of Neurological Disorders and Stroke.

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Antisense Oligonucleotide Therapy Against SCA3

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Abstract Spinocerebellar ataxia type 3 (SCA3), also known as Machado Joseph disease, is a dominantly inherited neurodegenerative disease that has remained, to date, untreatable. Recent advances in antisense oligonucleotide (ASO) technologies have reinvigorated the clinical potential of these drugs, with particular promise for the monogenetic neurodegenerative diseases, such as SCA3. This chapter discusses the basic and novel aspects of ASO therapy development against SCA3, with an emphasis toward ASO chemistry improvements and delivery approaches, a discussion on ASO risks, and overview of preparations for future clinical trials including assay development for target engagement assessments.

Keywords Antisense oligonucleotide · Gene silencing · SCA3 · MJD

1 Introduction

Spinocerebellar ataxia type 3 (SCA3), also known as Machado Joseph disease (MJD), is the most common autosomal dominant ataxia worldwide (Durr 2010; Gardiner et al. 2019). SCA3 has significant regional prevalence variations in which SCA3 represents close to half of autosomal dominant ataxias in Eastern Asia, Brazil, and Portugal, but less than 2% in Finland or Italy (Buijsen et al. 2019). SCA3 is characterized by a wide range of progressive motor impairments typically beginning in the third to fifth decade of life and SCA3 patients exhibit a rate of decline usually 10–15 years after symptom onset (Jacobi et al. 2015; Diallo et al. 2018). The progressive motor impairments observed in SCA3 patients are the result of loss in

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_16

motor and somatosensory nuclei spanning the spinal cord, brainstem, and into the striatum and deep cerebellar nuclei, with relative sparing of the olivary nuclei and cerebellar cortex (Rub et al. 2003, 2004, 2013; Fahl et al. 2015). SCA3 pathology also is found outside of the central nervous system (CNS), including muscle atrophy and areflexia, due to progressive peripheral neuropathy (Suga et al. 2014; Linnemann et al. 2016).

The genetic cause of SCA3 disease has been known for decades: caused by a polyglutamine repeat expansion in the coding region of ATXN3 (Kawaguchi et al. 1994). While advances are being made toward better understanding the SCA3 pathogenic pathways, including disruption of the ubiquitin proteasome system, autophagic dysfunction, overextended cellular stress pathways, and impaired DNA damage repair pathways, most published data suggest that SCA3 is caused by a toxic gain of ATXN3 function (previously reviewed by Matos et al. 2018; McLoughlin et al. 2020). Unlike many other polyglutamine neurodegenerative diseases, germline knockout of endogenous *Atxn3* in mice shows no overt phenotypes, suggesting that ATXN3 may not be an essential protein in mammals (Schmitt et al. 2007; Reina et al. 2010). Using a conditional mouse model of SCA3 to genetically turn off the CAG_{77} repeat expanded *ATXN3* gene early in the disease progression reverted the assessed disease phenotypes to wildtype states (Boy et al. 2009). Therefore, strategies to modify or directly reduce ATXN3 expression would likely be well tolerated in SCA3 patients. Recent advances in antisense oligonucleotide (ASO) technologies have reinvigorated the clinical potential of these drugs, showing therapeutic promise for this monogenetic neurodegenerative disease. This chapter will discuss basic and novel aspects of ASO therapy development for SCA3.

2 Antisense Oligonucleotide Therapy Targeting Strategies

ASO is a broad term for a short (12–50 nucleotides) sequence of single-stranded oligonucleotides that can bind RNA through Watson-Crick hybridization. Investigations for the use of ASOs as a therapeutic drug began in the late 1970s following the observation that mRNA hybridization to a complementary DNA sequence is not translated into a protein (Stephenson and Zamecnik 1978). Over the years, modifications to ASO sugar rings and phosphate backbones have improved their pharmacological properties. Briefly, 2′-sugar modifications improve binding affinity to target RNA, improve the ASO's safety profile, and increase their metabolic stability, whereas the phosphate backbone modifications impart nuclease protection and improve tissue distribution and cellular uptake by increasing protein binding (reviewed by Crooke et al. 2018; Bennett et al. 2019). Based on the target sequence and the chemical modifications to the ASO, the hybridized RNA molecule and ASO heteroduplex can modify the target protein's expression. ASOs targeting ATXN3 have been developed and tested in SCA3 cellular and animal models (Table 1) and can be broadly divided into two ASO functional mechanisms: RNase H1-independent and RNase H1-dependent mechanisms.

Table 1 Preclinical SCA3 studies using ASOs

ASO target; chemistry	Model	Molecular and phenotypic result	References
ATXN3 exon 9 and 10 skipping; PS-2′-O-Me	SCA3 patient-derived fibroblasts	Evidence of *ATXN3* exon 9 and 10 skipping while maintaining truncated ATXN3 ubiquitin-binding function	Evers et al. (2013)
	Wildtype C57BL/6J mice	Targeting endogenous *Atxn3*; ICV injection of ASOs leads to ~35% exon 9 and 10 splicing by qPCR 1 week after treatment	
ATXN3 truncation at 5′ of exon 10; PS-2′-O-Me	SCA3 patient-derived fibroblasts	Exon 10 splicing ASOs result in ATXN3 protein lacking C-terminus Truncated ATXN3 maintains ability to bind and cleave ubiquitin chains	Toonen et al. (2017)
	SCA3 YACQ84.2 hemizygote mice	ICV bolus of exon 10 ASO showed reduced CNS insoluble ATXN3 and decreased substantia nigra nuclear ATXN3 expression	
ATXN3 exon 8 and 9 skipping; PMO	SCA3 patient-derived fibroblasts	PMOs truncated similar proportion of ATXN3 as PS-2′-O-Me ASOs No evidence of toxicity by NONO paraspeckle protein expression with PMO treatment	McIntosh et al. (2019)
ATXN3 non-allele-specific targeting; PS-2′-MOE gapmer	SCA3 patient-derived fibroblasts	Suppression of mutant and WT ATXN3 protein expression in SCA3 fibroblasts by 80% after 72-hour treatment	Moore et al. (2017)
	SCA3 YACQ84.2 hemizygote mice	ICV bolus showed reduced pontine and deep cerebellar nuclei nuclear ATXN3 localization Reduced aggregated ATXN3 protein expression throughout CNS tissue	
ATXN3 targeting; PS-2′-MOE gapmer	SCA3 YACQ84.2 homozygote mice	Longitudinal assessment of ASO gapmer; sustained reduction of aggregated ATXN3 and rescue of motor impairment	McLoughlin et al. (2018)
ATXN3 targeting; PS-2′-MOE gapmer	SCA3 human embryonic stem cells	Reduced ATXN3 protein expression and aggregation in ASO-treated cells Decrease in p62 puncta after treatment	Moore et al. (2019)
ATXN3 targeting; PS-2′-MOE gapmer	SCA3 YACQ84.2 hemizygote mice	Rescue of Purkinje cell K_V channel expression and neuronal excitability by ASO treatment in 16-week-old mice	Bushart et al. (2020)

Abbreviations: *2′-MOE* 2′-O-methoxyethyl; *ASO* antisense oligonucleotide; *CNS* central nervous system; *ICV* intracerebroventricularly; K_V voltage-gated potassium; *NONO* non-POU domain containing octamer binding; *PMO* phosphorodiamidate morpholino; *PS-2′-O-Me* phosphorothioate 2′-O-methyl; *qPCR* quantitative polymerase chain reaction; *SCA3* spinocerebellar ataxia type 3

2.1 *Targeting the ATXN3 Transcript for RNA Degradation: RNase H1-Dependent Mechanism*

ASOs that are RNase H1-dependent have a gapmer design (Fig. 1). The most commonly employed gapmer design in CNS preclinical studies is an ASO with five 2′-*O*-methoxyethyl (2′-MOE)-modified nucleotides on the 5′- and 3′-termini and eight to ten unmodified oligodeoxynucleotides in the central region to support the cleavage of the RNA strand in an RNA:DNA hybrid via the RNase H1 enzyme (Wu et al. 1999). To improve nuclease resistance and enhance cellular uptake, all nucleotides can be modified with a phosphorothioate (PS) backbone (Bennett and Swayze 2010). The catalytic RNase H1 enzyme can be found both in the nucleus and the cytoplasm, allowing for targeted cleavage throughout the cell (Liang et al. 2017). As pre-mRNA splicing occurs in the nucleus before migrating out into the cytoplasm, the nuclear RNase H1 activity allows for a broad ASO targeting landscape into the intronic sequences of disease transcripts.

Recent studies performed in SCA3 cell and mouse models showed potent evidence for non-allele-specific ASO gapmers to target *ATXN3* and subsequently

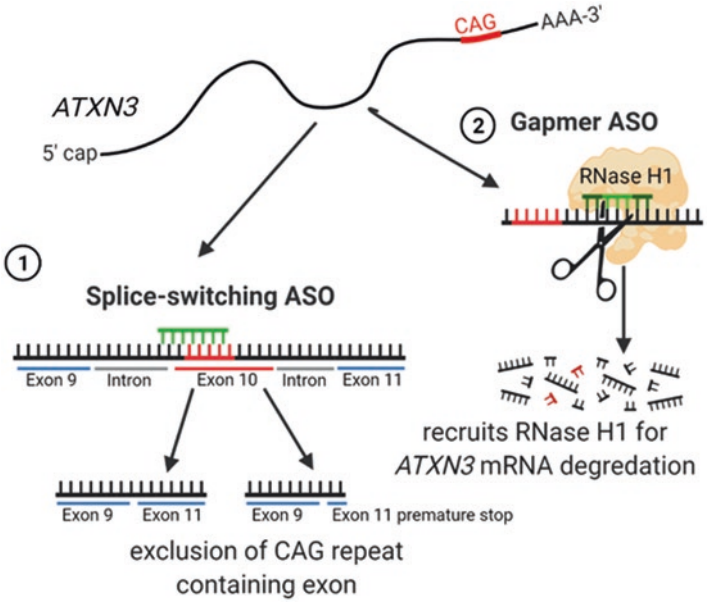

Fig. 1 Antisense oligonucleotides' mechanisms of action against ATXN3. Through sequence-specific targeting, preclinical evidence in SCA3 models have been shown to disrupt mutant ATXN3 expression through two different ASO mechanisms of action: (1) ASOs can interfere with the endogenous splicing machinery controlling the inclusion or exclusion of interest exons such as the CAG repeat exon 10 in *ATXN3* using a splice-switching ASO, or via (2) gapmer ASOs that can recruit RNase H1 for a sequence-specific degradation of the *ATXN3* target mRNA. (Image created with Biorender.com)

reduce mutant ATXN3 protein. In human cell lines, gapmer ASOs were shown to reduce mutant *ATXN3* expression up to 95% in human SCA3 patient-derived fibroblasts and up to 75% in human SCA3 embryonic stem cells after a 72-hour treatment (Moore et al. 2017, 2019). In vivo assessment of these ASOs were performed in SCA3 mouse models: a transgenic Yeast Artificial Chromosome (YAC) MJD-Q84.2 mouse expressing the full-length human *ATXN3* gene and the Cytomegalovirus (CMV) MJD-Q135 mouse expressing the most common human *ATXN3* cDNA isoform (Moore et al. 2017). In this cross-sectional in vivo study, SCA3 mice were intracerebroventricularly (ICV) injected with anti-*ATXN3* gapmer ASOs and brain tissue was collected 4 weeks after treatment. In the YAC MJD-Q84.2 mouse model, many of the ASOs achieved safe and widespread delivery throughout the mouse CNS and efficient silencing of human mutant ATXN3 throughout SCA3 vulnerable brain regions (Moore et al. 2017). In contrast to the robust target engagement in the YAC Q84.2 mice, ASOs did not silence mutant *ATXN3* in the CMV MJD-Q135 mice expressing a single *ATXN3* cDNA isoform. However, endogenous *Atxn3* levels were equally silenced between the YAC and CMV mouse models using ASO-5. Immunofluorescence studies confirmed that the lack of ASO efficacy toward the mutant ATXN3 transcript was not due to delivery issues in the CMV model, as broad ASO expression was observed throughout the brain. The authors propose that the decreased potency of ASO disease gene targeting in the CMV Q135 mouse may be due to differences in RNA dynamics between the two models. The lack of pre-mRNA splicing leads to a reduced nuclear dwell time of this small cDNA (~2 kb; GenBank accession number U64820.1) in the CMV Q135 mouse model compared to the YAC Q84.2 mice expressing a full-length human ATXN3 disease gene (~250 kb human MJD1-YAC).

To assess whether sustained mutant *ATXN3* suppression by gapmer ASOs can rescue key molecular, pathological, electrophysiological, and behavioral hallmarks of the disease, the McLoughlin and Paulson labs further performed a longitudinal preclinical study in the YAC Q84.2 mouse model (McLoughlin et al. 2018). Key molecular and pathological results showed anti-*ATXN3* ASO gapmers sustained reduction in the mutant ATXN3 protein up to 8 weeks after a single ICV ASO treatment and alleviated ATXN3 nuclear accumulation at least 14 weeks after treatment. Functionally, at the cellular level, anti-*ATXN3* ASOs rescued previously reported defects in cerebellar Purkinje neuron firing frequency and afterhyperpolarization in these mice (Shakkottai et al. 2011). Further assessments at the cellular level showed an association of SCA3 Purkinje neuron excitability with reduced voltage-gated potassium (K_V) channel expressions that are rescued by ASO treatment (Bushart et al. 2020). At the organism level, repeated ICV ASO treatments in SCA3 mice rescued motor function back to the abilities matching wildtype littermates (McLoughlin et al. 2018). The motor rescue in these animals is arguably the most promising result as future ASO clinical trial endpoints will most likely rely, at least in part, on motor improvement measured through clinical assessments such as the Scale for Assessment and Rating of Ataxia (SARA) (Schmitz-Hubsch et al. 2006).

2.2 ASOs Targeting RNA Processing: RNase H1-Independent Mechanism

When all the ribose sugar 2′ positions include a modification, the ASO is referred to as "fully modified." Such fully modified ASOs are unable to support RNase H activity. Rather, these fully modified ASOs can modulate RNA processing. Less common targeting strategies utilizing fully modified ASOs include the following: targeting polyadenylation sites to modify stability (Vickers et al. 2001), inhibition of nonsense-mediated decay factor assembly on the targeted mRNA (Nomakuchi et al. 2016), translational inhibition by blocking polymerase reading activity (Bennett and Swayze 2010), and blocking endogenous miRNA activity to increase target protein translation (Koval et al. 2013; Butovsky et al. 2015). The most well-established approach utilizing fully modified ASOs is splice switching, where the ASO complimentarily binds to a splice donor or acceptor site to redirect the splicing activity of the transcript of interest.

Nusinersen was the first US Food and Drug Administration (FDA)-approved CNS-delivered splice-switching ASO for the treatment of spinal muscular atrophy (SMA) (Hua et al. 2010; Finkel et al. 2016; Chiriboga 2017). SMA is caused by loss-of-function mutations in the *Survival Motor Neuron 1* (*SMN1*) gene and the levels of remaining SMN protein directly related to SMN onset and disease progression. The treatment of Nusinersen alters the splicing of an *SMN2* transcript, a second highly homologous copy of *SMN1,* to increase the production of the functional SMN protein (Hua et al. 2010). Intrathecal delivery of Nusinersen to infants led to safe, well-tolerated, widespread delivery throughout the spinal cord and brain regions of patients (Finkel et al. 2016). Phase III clinical studies of Nusinersen reported lifespan extension as well as the remarkable quality of life improvements, whereby treated infants were able to reach motor milestones that were unattainable by placebo control patients (Finkel et al. 2016).

In the context of SCA3 disease, the majority of fully 2′-modified ASOs have been employed to alter ATXN3 splicing (Fig. 1). Two key papers toward this ASO targeting strategy have come from the van Roon-Mom group. In the Evers et al. (2013) paper, authors tested the potential of PS-2′-O-Me splice-switching ASOs to exclude *ATXN3* exon 9 and the CAG-containing exon 10. In SCA3 patient-derived fibroblasts, they report transcriptional splicing efficiency that resulted in a modified ATXN3 protein that lacked the polyglutamine repeat while still maintaining the Josephin domain, the nuclear export signal, the nuclear localization signal, and the functional ubiquitin interacting motifs (UIMs) (Evers et al. 2013). They demonstrated the in vivo potential and acute tolerability of these splicing ASOs by ICV delivering to wildtype mice. This treatment resulted in over 35% of mouse ATXN3 exons 9–10 skipped in cerebellar tissue 7 days after treatment. Using a similar targeting strategy, Toonen and colleagues tested the efficacy of exon 10 skipping using PS-2′-MOE ASO that leads to a truncated ATXN3 protein lacking the polyglutamine repeat and the UIM3 domain in exon 11 (Toonen et al. 2017). ICV treatment with these exon 10 targeting ASOs in the hemizygote transgenic YAC MJD-Q84.2

mouse resulted in the truncation of ~40% of ATXN3 protein in the brainstem, cerebellum, and cortex 2.5 months after treatment relative to control treated animals. They also report that ASO treatment reduced nuclear ATXN3 accumulation in the substantia nigra, a vulnerable brain region in SCA3 disease.

Similar to fully modified 2′-MOE and 2′-O-Me ASOs, ASOs with morpholino chemical modifications do not allow for RNase H1 to bind. The phosphorodiamidate morpholino (PMO) modification has been shown to increase metabolic stability while decreasing protein binding; therefore, PMOs are thought to be less toxic and more efficient as they are retained longer in the system (Bennett et al. 2019). In 2019, McIntosh and colleagues found similar efficiency of the previously published PS-2′-O-Me splice-switching ASOs relative to their morpholino PMO-modified ASOs targeting exon 10 (McIntosh et al. 2019). A report from Stanley Crooke's group noted PS backbone ASO recruitment in paraspeckles is potentially cytotoxic (Shen et al. 2014). McIntosh and colleagues build on this study and show in SCA3 patient fibroblasts that the PS backbone ASO induces paraspeckle formation whereas PMO ASOs do not. A recent review clearly outlines different ASO backbone absorption, distribution, metabolism, and excretion characteristics (Shadid et al. 2021). Furthermore, direct comparison studies of splice-switching MOE and PMO ASOs in SMA models highlighted these advantages and disadvantages to each chemistry, including the potential to cross the immature blood brain barrier (BBB), persistence in treated tissues, and dose tolerability (Sheng et al. 2020).

ASOs modifying ATXN3 splicing are very promising as they show proof of concept data that polyglutamine skipping both is tolerated and potent in targeted SCA3 vulnerable brain regions of mice. This approach alleviates concerns for loss of function, as truncation at exon 10 preserves overall ATXN3 transcript and protein expression levels as well as maintains ubiquitin functions and nuclear signals. However, SCA3 literature notes that naturally occurring isoforms lacking the UIM3 have been shown to have higher rates of deubiquitination relative to the major isoform containing the exon 11 localized UIM3 (Weishaupl et al. 2019). It would be interesting to determine how these truncated ASO-spliced ATXN3 proteins behave when this ratio of 2UIM:3UIM ATXN3 is increased in vivo. Additionally, a recent study by the Todi lab found that UIM addition, in general, enhances toxicity in SCA3 fly models (Johnson et al. 2019). Follow-up studies in mice should include assessment of whether ASO skipping of exons containing the CAG repeat and the UIM3 alters localization, function, or aggregation throughout the CNS. Finally, longitudinal experiments should seek to evaluate whether polyglutamine skipping improves motor and electrophysiological disease phenotypes in SCA3 mice, as was previously assessed using anti-*ATXN3* gapmer ASOs.

3 ASO Delivery Methods to the CNS

Although the ASO chemical modifications have improved over the past decades, the treatment of neurological disease is still challenged by the delivery of ASOs in neurological disorders. As SCA3 affects multiple brain regions spanning the brainstem, cerebellum, spinal cord, and striatum, an effective gene silencing approach for SCA3 will likely require widespread delivery to the many vulnerable brain regions in SCA3 patients. Additionally, all of the studies to date consider ASO therapeutic efficacy on neuronal CNS health, while there is growing evidence of non-neuronal contributions to SCA3 pathogenesis that may be a relevant consideration for preclinical development (previously reviewed by McLoughlin et al. 2020). Therefore, the development of any therapeutic intervention will need to consider target engagement across multiple cell types. In this section, we will discuss current delivery approaches as well as those that are currently in development.

3.1 Direct Invasive CNS Delivery

The success of intrathecal ASO delivery programs, such as the previously discussed Nusinersen ASO for SMA patients, has established this invasive direct route of administration as the standard method for CNS delivery. For many diseases of the CNS, delivering efficient drugs to affected regions or cells at appropriate concentrations is a major hurdle. The biggest challenge is the blood brain barrier, which restricts entry of drugs based on size, solubility, and charge (Bennett et al. 2017). ASOs fit into the category of drugs that have limited ability to cross the blood–brain barrier, with an example of a systemic delivery of ASOs in Alzheimer's disease mice reporting less than 1% of oligonucleotides reaching the brain (Banks et al. 2001). Therefore, most neurodegenerative disease ASO studies administer drug directly into the brain through ICV or intrathecal injections. Of note, all preclinical SCA3 animal studies have been directly administered ASOs to the CNS via the ICV route (Evers et al. 2013; Moore et al. 2017; Toonen et al. 2017; McLoughlin et al. 2018; Kourkouta et al. 2019). Once inside the CNS, ASOs have been shown in multiple small and large animal models as well as in human clinical trials to widely distribute throughout the brain and spinal cord. A review of the biodistribution of Malat1-ASO delivered ICV in mice, intrathecal in rats, and intrathecal in non-human primates provides evidence that both routes provide a central delivery that has widespread distribution throughout the CNS (Jafar-Nejad et al. 2021). Intrathecal ASO delivery has already been implemented in human clinical trials for SMA and amyotrophic lateral sclerosis (ALS) with safe and tolerable results (Miller et al. 2013; Chiriboga 2017; Ly and Miller 2018). However, news from the recently halted Roche anti-*Huntingtin* (*HTT*) non-allele-specific gapmer ASO clinical trial in Huntington's disease does call for some investigation of delivery method review. Among the many potential mechanisms underlying the negative outcomes of the

anti-*HTT* ASO trial, the possibility that high doses of ASOs needed to reach deep brain regions, such as the striatum, may have inadvertently contributed to overdosing of other brain tissues. Answers from this program may inform future SCA3 clinical delivery routes, as SCA3 vulnerable brain regions also require deep tissue targeting.

Alternatives to the broad intrathecal administration approach for ASOs may be through packaging in nanocarriers, liposomes, exosomes, and spherical nucleic acids (SNAs) (Roberts et al. 2020). An example of this nanoparticle delivery technology is being developed through Exicure, who are testing their proprietary spherical nucleic acid (SNA) technology to deliver use ASO-based therapy for SCA3. They publicly presented screening data that showed up to a 95% reduction in the ATXN3 transcript in patient neurons but have yet to show potential for CNS delivery in SCA3 models.

3.2 Indirect Noninvasive CNS Delivery

We will likely see the implementation of novel delivery devices such as the brain shuttle model that may enable less invasive intravenous injections in the future. Alternative ASO delivery routes to the CNS include ASOs conjugated to lipids, GalNAc antibodies, aptamers, and peptides (recently reviewed in *Nature Reviews Drug Therapy* by Roberts et al. 2020). Currently, there is no literature support for SCA3 ASO delivery through these conjugation mechanisms. Alternative routes for CNS ASO delivery may also be possible through intranasal injections. Intranasal injections have been used for many other drugs as a way to bypass the blood brain barrier through uptake by the olfactory and trigeminal neural pathways (Illum 2000). Intranasal ASO injections, although limited in literature support, have shown some encouraging delivery results that yield CNS target mRNA silencing. One promising study from the Parkinson's disease field showed that intranasal administration of an ASO covalently bound to a triple inhibitor of monoamine transporter efficiently delivered monoamine neurons and inhibited serotonin transporter (SERT) expression and function (Alarcon-Aris et al. 2018). A recent publication in *Nature Biotechnology* demonstrates a new formulation method for ASOs to cross the BBB by cholesterol-conjugating DNA/RNA heteroduplex oligonucleotides (Nagata et al. 2021).

4 Limiting ASO Off-Targets

In addition to choosing the appropriate ASO chemical modifications and delivery method, another important consideration for ASO development is target specificity. Whereas RNase H1 ASOs can target intronic, exonic, and untranslated region (UTR) sequences of the gene target, splice-switching ASOs are limited to

targeting nucleotides that normally enhance or silencing splicing in the target gene. Diligence in off-target assessment both through computational prediction and quantitative polymerase chain reaction (qPCR) assessment of potential off-targets is necessary for progression to the clinic. In general, ASOs with one or two mismatches to other mRNAs should be excluded entirely. A recent study by Scharner and colleagues detailed the process to assess hybridization-dependent mis-splicing of unintended targets (Scharner et al. 2020). To date, neither the SCA3 splice-switching ASOs nor gapmer ASOs have published evidence of a clean off-target profile (Toonen et al. 2017; McLoughlin et al. 2018), although ASO sequences were published and are available for computational review. As current delivery methods are directly delivered to the CNS, future SCA3 ASO studies should explore the off-target potential of single and double nucleotide mismatch targets from human CNS-expressed transcripts.

5 Hurdles for Moving SCA3 ASO Application to the Clinic

The preclinical efficacy and tolerability data from anti-*ATXN3* ASO animal model studies strongly support the continued development of SCA3 ASOs. As there are no reported humans that are haploinsufficient for ATXN3, it is not yet clear how tolerable non-allele-specific ASO targeting in humans will be. Thus, before clinical trials commence, ASO tolerability and toxicity studies of ATXN3 silencing in large animal models, such as non-human primates, need to be completed. If results in these large animal studies show concern, allele-specific ASO targeting of known single nucleotide polymorphisms (SNPs) may be prioritized. As there are multiple haplotypes in disease (Costa et al. 2019; Ramos et al. 2019), a priority will be targeted to ATXN3 SNPs that reside in *cis* with the CAG-expanded allele (Melo et al. 2022). An example of this approach has recently been demonstrated in SCA3-induced pluripotent stem cell (iPSC)-derived neurons (Hauser et al. 2022). However, the main limitation of targeting allele-specific-targeting SNPs is that no one SNP is present in *cis* on the CAG-expanded allele across the human SCA3 patient population. Rather there is a predominant A^{669}-C^{987}-A^{1118} haplotype that is found in over 70% of SCA3 patients (Gaspar et al. 2001). Therefore, it is likely that numerous allele-specific ASOs will need to be developed in order to target all of the human SCA3 patient population.

As SCA3 is a slowly progressing neurodegenerative disease, natural history studies will be essential to define the timing for the first administration of the disease-modifying treatment. These natural history studies will likely need to define not just the phenotypic changes in SCA3 patients, but also the molecular changes that correlate with SCA3 disease pathogenesis. Currently, the SARA score is the most used method to track disease progression in SCA3 patients; however, this scale is limited by the sensitivity across patients over time (Schmitz-Hubsch et al. 2006). For example, the average annual SARA score increase in SCA3 patients is only 1–2 points and a proposed clinical trial will need 202 patients to show a 50% reduction

in progression of SARA score over a 1-year trial (Jacobi et al. 2015). The ability to recruit and retain that number of SCA3 patients for an effective ASO trial would be challenging.

SCA3 biofluid biomarker studies may provide more acute and sensitive molecular measurements to assess response to anti-*ATXN3* ASO therapy. Recently, sensitive immunoassays against human mutant ATXN3 expression have been developed for the evaluation of mutant protein in SCA3 cerebrospinal fluid (CSF), plasma, and urine samples (Prudencio et al. 2020; Hubener-Schmid et al. 2021; Koike et al. 2021). While the less-invasive assessment of mutant ATXN3 in plasma and urine may help track disease progression, CSF mutant ATXN3 expression levels will be essential in defining target engagement of anti-*ATXN3* ASOs. For studies using splice-switching ASOs that remove the mutant exons but preserve the expression levels of total ATXN3, it would be helpful to develop a sensitive detection assay for total ATXN3 expression in biofluids. Other strong clinical biomarkers, such as neurofilament light (NfL), can define a molecular read-out for neuronal health. Researchers have assessed NfL levels in SCA3 patient CSF and plasma samples and found NfL levels positively correlate with disease progression (Wilke et al. 2018; Li et al. 2019; Costa et al. 2020). A recent review highlighted other biofluid biomarkers that are in development, any of which may be considered in a comprehensive biofluid biomarker score for clinical endpoints once validated in large SCA3 natural history studies (Brooker et al. 2021).

In conclusion, many in the field believe this dominantly inherited spinocerebellar ataxia to be among the lowest hanging fruit in terms of neurodegenerative disease gene-altering therapy due to the monogenic nature of the disease and the prevalence across all dominantly inherited ataxias. As this chapter detailed, while ASO gene-altering strategies targeting the affected gene may seem straightforward, many hurdles have had to be overcome. In part to the advances in ASO chemistry and delivery approaches, ASO therapy for SCA3 holds a robust potential for clinical translation. To date, SCA3 preclinical studies have established the preliminary efficacy of splice-switching ASOs and gapmer ASOs in SCA3 cell and mouse models. These results warrant final preclinical development that addresses phenotypic efficacy and safety in small and large animal models to proceed to future SCA3 ASO clinical trials.

Acknowledgments This work was supported in part by grants from the NIH/NINDS R21-NS111154, R01-NS122751, and U01-NS106670.

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Spinocerebellar Ataxia Type 7: From Mechanistic Pathways to Therapeutic Opportunities

Pawel M. Switonski and Albert R. La Spada

Abstract Spinocerebellar ataxia type 7 (SCA7) is a cerebellar and retinal neurodegenerative disease caused by a CAG/polyglutamine (polyQ) expansion mutation in the ataxin-7 (ATXN7) gene. PolyQ-expanded ataxin-7 protein interferes with the histone modification activity of the STAGA co-activator complex and consequently alters the expression of STAGA-regulated genes. In the SCA7 pathogenic cascade, epigenetic dysregulation of the DNA repair interactome, combined with increased oxidative stress, leads to the accumulation of DNA damage and PARP1-mediated depletion of nicotinamide adenine dinucleotide (NAD+) levels. Subsequent breakdown of the SIRT1/NAD+—PPARγ/PGC-1α transcriptional regulatory axis, arising from NAD+ depletion, results in altered expression of calcium homeostasis genes, culminating in neuronal dysfunction and death. Here, we describe the current mechanistic understanding of SCA7, highlighting recent advances in this field. Based upon our understanding of the cellular and molecular basis of SCA7 disease pathogenesis, we delineate both known and prospective therapeutic targets and treatment strategies that could ameliorate polyQ-expanded ataxin-7 neurotoxicity, including recent progress toward an antisense oligonucleotide therapy directed against ATXN7 mRNA. In the near future, preclinical and clinical exploration of these therapeutic opportunities may yield a highly effective treatment for SCA7.

Keywords Ataxin-7 · Polyglutamine · Repeat expansion · Nicotinamide adenine dinucleotide · Sirtuin-1 · PARP1 · PPARs · Transcription · DNA damage · Calcium · Antisense oligonucleotide

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_17

1 Introduction

Spinocerebellar ataxia type 7 (SCA7) is a neurodegenerative disease, typically affecting patients in mid-late adulthood (Garden and La Spada 2008). Due to prominent anticipation, disease onset is occasionally shifted to earlier ages, with many reports describing adolescent, early childhood, and even infantile-onset SCA7 cases (Donis et al. 2015; Michalik et al. 2004). SCA7 is a relatively rare disorder with a global prevalence estimated at 1:300,000. However, local founder effects observed in Mexican, Scandinavian, and African populations increase the total number of SCA7 patients significantly above this worldwide average (Smith et al. 2015; Magaña et al. 2014; Jonasson et al. 2000). Together with other genetic ataxias, SCA7 falls into the category of autosomal dominant cerebellar ataxias (ADCAs), characterized by cerebellar dysfunction and other neurological symptoms. SCA7 patients suffer from coordination deficits, walking difficulties, speech impairment, and oculomotor abnormalities. Contrary to other ADCAs, SCA7 has an additional retinal component in the form of a progressive cone-rod dystrophy that in many instances leads to total blindness (Michalik et al. 2004).

Similar to other neurodegenerative disorders, SCA7 is characterized by a selective pattern of neuronal dysfunction and cellular loss. The most prominent degenerative changes are observed among Purkinje cells (PCs) of the cerebellar cortex, inferior olive neurons of the medulla oblongata, and photoreceptors in the retina. Neurons in other brain regions are relatively spared, though moderate changes can occur in the cerebral cortex, basal ganglia, midbrain, and thalamus (Rüb et al. 2008).

SCA7 is a dominantly inherited genetic disease that is caused by excessive elongation of a CAG trinucleotide repeat tract in the *ATXN7* gene (Lindblad et al. 1996). As observed for other repeat expansion disorders, when the repeated tract exceeds a certain length threshold (~38 CAGs in SCA7), affected individuals exhibit pathology and develop neurological signs and symptoms. Shorter CAG expansions can be associated with a less severe phenotype and incomplete clinical penetrance, including mild cerebellar signs and ALS-like upper and lower motor neuron disease (Cluse et al. 2021). The CAG repeat expansion is located in the coding region of the gene and consequently is translated into an elongated polyglutamine (polyQ) domain in the resulting ataxin-7 protein (Michalik et al. 2004). Analysis of a polyQ domain present in mutant proteins associated with SCA7 and other polyQ diseases helped formulate the hypothesis that a gain of new toxic functions by causative proteins triggers a molecular cascade that culminates in neuronal dysfunction and death (Paulson et al. 2017).

2 SCA7 Molecular Cascade

2.1 Ataxin-7 Function

Understanding the molecular basis of SCA7 has been a topic of extensive research since the mid-1990s, when a series of studies established the CAG repeat expansion mutation as the root cause of the disease (David et al. 1997). Subsequent research identified ataxin-7 as a transcription cofactor that epigenetically modulates gene expression. Mass spectrometry data generated from yeast and mammals revealed that ataxin-7 (and its yeast ortholog Sgf73) is a member of the multiprotein high-molecular-weight SPT3-TAF9-GCN5 acetyltransferase (STAGA) complex (Palhan et al. 2005; Helmlinger et al. 2004; Sanders et al. 2002). Mammalian STAGA possesses both histone acetyltransferase (HAT) and deubiquitinase (DUB) activity, governed by GCN5 and USP22, respectively. Both of these activities, via H3K9ac modification (HAT) and H2Bub removal (DUB), are performed by STAGA at active genes to secure optimal initiation and elongation of transcription (Baker and Grant 2007). Although the exact role of ataxin-7 in the STAGA complex is not fully understood, Lee and colleagues have shown that yeast Sgf73 is required to recruit DUB module components and anchor them to the rest of the SAGA (equivalent of mammalian STAGA) complex (Lee et al. 2009). Only when associated with SAGA can the DUB module carry out its proper functions.

Further evidence for the transcriptional regulatory activity of ataxin-7 comes from our work on SCA7 retinal degeneration in transgenic mice, which we created to accurately recapitulate all the cardinal features of the cone-rod dystrophy phenotype observed in human patients (La Spada et al. 2001). To determine the mechanism of photoreceptor degeneration displayed by SCA7 model mice, we examined the cone-rod homeobox protein (CRX), a transcription factor that is predominantly expressed in retinal neurons. Using yeast two-hybrid assays and through a series of co-immunoprecipitation experiments, we documented that ataxin-7 physically and functionally interacts with CRX and that polyQ-ataxin-7 prevents CRX-dependent gene expression in retinal photoreceptors (La Spada et al. 2001), as both factors co-occupy the promoter and enhancer regions of CRX-regulated retinal genes (S. Chen 2003). Further investigation indicated that ataxin-7 acts as a mediator between the STAGA complex and CRX to facilitate expression of certain retinal genes (Palhan et al. 2005).

2.2 PolyQ Ataxin-7 Toxicity

Where does polyQ expansion toxicity come from? In retina, the inability of CRX to bind to its consensus DNA sequences in the presence of polyQ-ataxin-7 suggests that transcriptional interference of photoreceptor-specific genes is the key factor leading to cone-rod dystrophy (La Spada et al. 2001) (Fig. 1). Similarly,

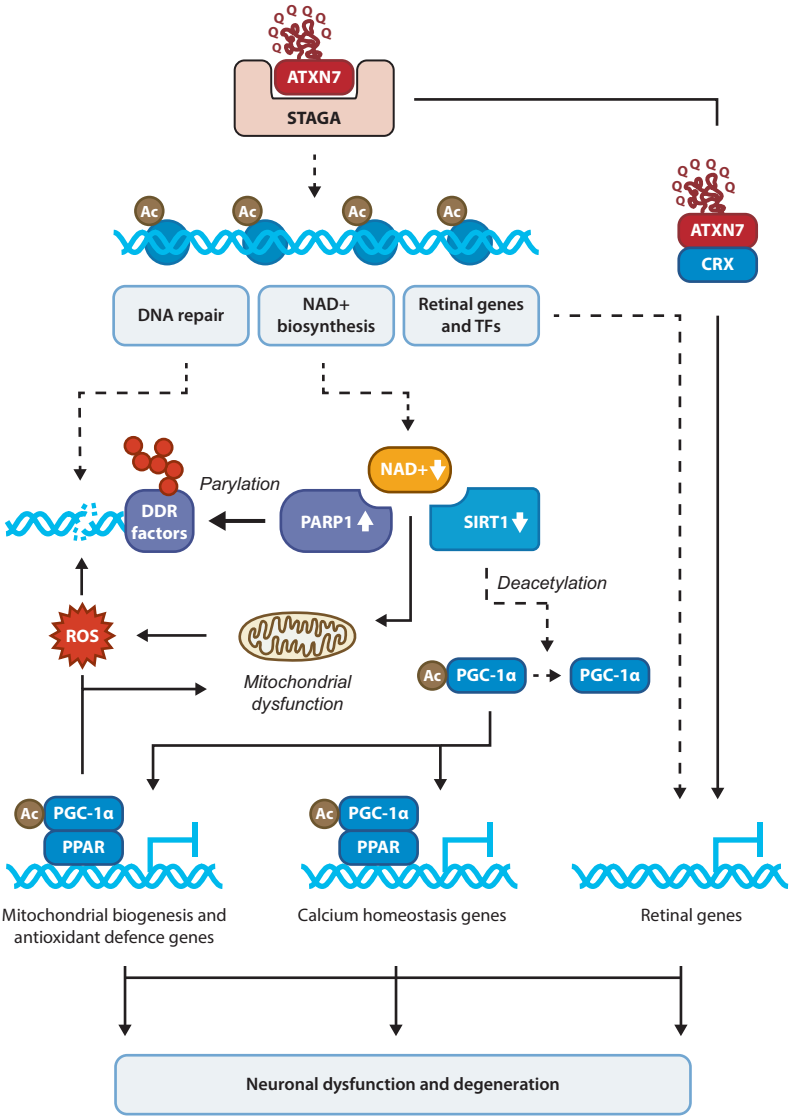

Fig. 1 Transcription dysregulation: a unifying model for SCA7 disease pathogenesis. Polyglutamine-expanded ataxin-7 disturbs histone acetyltransferase and histone deubiquitinase activities of the STAGA co-activator complex, and this epigenetic dysregulation interferes with the CRX-dependent transcription of retinal genes. Polyglutamine-expanded ataxin-7 may also induce aberrant epigenetic regulation of genes involved in NAD+ biosynthesis and response to DNA damage in the cerebellum. An accumulation of DNA damage results in PARP1 hyperactivation and overutilization of NAD^+, which in turn disrupts SIRT1 ability to activate PGC-1α-dependent transcriptional programs. Subsequent transcriptional dysregulation is the cause of calcium dyshomeostasis, mitochondrial dysfunction, and increased oxidative stress, which together combine to cause neuronal dysfunction, ultimately culminating in neuron cell death. Dashed lines indicate diminished regulatory activities of a parent molecule or biochemical process

downregulation of the retinal transcription factors Nrl, Crx, and Nr2e3 and target genes involved in rod visual phototransduction and morphogenesis correlates with retinal disease phenotypes in SCA7 transgenic mice (Abou-Sleymane et al. 2006). Further research revealed that incorporation of polyQ-expanded ataxin-7 into STAGA results in formation of a HAT-deficient complex. In vitro experiments performed on free histones and nucleosomes indicate that polyQ-ataxin-7 firmly binds to the Gcn5 catalytic core of yeast SAGA, causing a potent reduction in the acetyltransferase activity (Burke et al. 2013). Additionally, in the presence of polyQ-ataxin-7, SAGA shows insufficient recruitment of other protein complex components, such as Ada2, Ada3, and TAF12, which also negatively affects HAT module activity (McMahon et al. 2005). This dominant-negative model of polyQ-ataxin-7 toxicity has been supported by chromatin immunoprecipitation (ChIP) analysis performed on retinas of symptomatic PrP-SCA7-92Q transgenic mice, which show significant reduction in the histone H3 lysine 9 acetylation (H3K9ac) mark in the promoter regions of CRX-dependent genes (Palhan et al. 2005). Similarly, H3K9ac epigenetic dysregulation of promoters and enhancers of retinal genes, including rhodopsin and cone opsins, is observed in SCA7 266Q knock-in mice (Niu et al. 2018). Interestingly, STAGA containing polyQ-ataxin-7, when isolated from the retina of R7E SCA7 mice, was found to promote substantial histone 3 hyperacetylation and accompanying aberrant chromatin decondensation (Helmlinger et al. 2006a, b), indicating that polyQ-ataxin-7 incorporation into the STAGA complex can have both gain-of-function and loss-of-function effects, as has been observed in SCA1 (Lim et al. 2008). Similarly, using the ChIP-seq approach, we recently identified >700 genes with increased H3K9 promoter acetylation in the cerebellum of SCA7 266Q mice (Switonski et al. 2021).

The negative impact of polyQ ataxin-7 on USP22 and its DUB activity has been reported in HEK293T cells and human astrocyte cell culture models. Although enzymatic activity of the DUB module is the same regardless of whether normal ataxin-7 or polyQ-ataxin-7 is recruited to STAGA, polyQ-ataxin-7 and its DUB module partners are readily sequestered away into insoluble inclusions (Lan et al. 2015; McCullough et al. 2012; Yang et al. 2015). This sequestration may disrupt STAGA and remove its occupancy from target promoters, resulting in reduced deubiquitination of histone H2B. In line with this model, significant increases in global H2B ubiquitination levels have been detected in the cerebellum of another SCA7 transgenic mouse model (Lan et al. 2015).

2.3 Dysregulation of the SIRT1/NAD+—PPARγ/PGC-1α Regulatory Axis

Consistent with ataxin-7 involvement in transcriptional and epigenetic modulation, we, and others, have noted global transcription dysregulation in SCA7 cell culture and mouse models using unbiased RNA-seq and ChIP-seq techniques. Transcriptome

profiles of SCA7-140Q knock-in mouse cerebella reveal potent dysregulation of key genes regulating cGMP and phosphatidylinositol signaling pathways, together with synaptic long-term depression of Purkinje cells. Interestingly, a significant portion of these downregulated genes is also affected in SCA1 and SCA2 model mice, signifying the importance of these cellular processes in cerebellar degeneration (Niewiadomska-Cimicka et al. 2021). Furthermore, fxSCA7 92Q transgenic mice show transcriptional dysregulation of genes involved in calcium homeostasis, affecting calcium related conductance, endoplasmic reticulum calcium storage, and phosphatidylinositol signaling (Stoyas et al. 2020). In symptomatic mice, synergistic reduction in calcium ions and downregulation of the large-conductance calcium-activated potassium (BK) channel severely impacted Purkinje cell membrane excitability, resulting in extensive spiking irregularities and subsequent neurodegeneration. Transcription factor binding site analysis performed on promoters of differentially expressed genes identified in this study reveals significant enrichment of binding sites for peroxisome proliferator activated receptor gamma (PPARγ) and hypoxia-inducible factor 1 (HIF1) (Stoyas et al. 2020). The observed overrepresentation of the PPARγ binding motifs implies the involvement of sirtuin 1 (SIRT1) in SCA7 disease pathogenesis. SIRT1 is a NAD+-dependent regulatory deacetylase engaged in a variety of biological processes, including metabolism, aging, and nervous system function (Lin et al. 2005). Among other targets, SIRT1 positively regulates PGC-1α, which is the potent PPARγ coactivator controlling expression of genes associated with mitochondrial biogenesis, energy metabolism, and calcium homeostasis (Rodgers et al. 2008). Cerebellar tissue of symptomatic SCA7 mice displays significant hyperacetylation of PGC-1α accompanied by a marked reduction in NAD+ levels, consistent with impaired SIRT1 enzymatic activity. Moreover, genetic overexpression of SIRT1 rescues the calcium flux deficits and neurological disease phenotypes in two distinct SCA7 mouse models—fxSCA7 92Q and SCA7 266Q (Stoyas et al. 2020). Depletion of NAD+ levels was also observed in the nuclear and mitochondrial fractions of human neuroprogenitor cells (NPCs) derived from SCA7 patient induced pluripotent stem cells (iPSCs) (Ward et al. 2019). Reduction in mitochondrial NAD+ in SCA7 may contribute to impaired energy metabolism and abnormal mitochondrial function, manifested as decreased oxygen consumption and respiration in SCA7 266Q mice. Additionally, Purkinje cells of SCA7 mice display abnormal mitochondrial network structure with enlarged individual mitochondria (Ward et al. 2019). These observations, together with the evidence of bioenergetics defects documented in the brains of SCA7 patients as well as unbiased bioinformatic clustering analysis, support the notion that mitochondrial dysfunction is a defining feature of SCA7.

2.4 PARP1 Hyperactivation and Increased DNA Damage

There are at least two possible explanations for reduced NAD+ levels documented in cellular and mouse models of SCA7. First, perturbations in NAD+ biosynthesis pathways, namely dysregulation of the kynurenine pathway and reduced expression of NMNAT1-3 observed in SCA7 NPCs, suggest that NAD+ production struggles to meet all demands (Ward et al. 2019). This dysregulation is presumably caused by polyQ-ataxin-7-induced transcriptional alterations. Second, reduction in NAD+ levels may result from PARP1 hyperactivation (Fang et al. 2014). PARP1 consumes large amounts of NAD+ to catalyze polyADP ribosylation modification onto itself and other protein partners participating in the DNA damage response (Ray Chaudhuri and Nussenzweig 2017). Increased levels of PARP1 in SCA7 266Q mouse cerebella, together with increased signal from poly(ADP-ribose) in SCA7 patient post-mortem cerebellum, provide strong evidence for elevated PARP1 activity and subsequent NAD+ hyperutilization (Stoyas et al. 2020) (Fig. 1). The concept of SIRT1/PARP1 competition over their common cofactor is reinforced by observations showing that genetic deletion of PARP1 significantly increases NAD+ levels and consequently promotes SIRT1 activity (Bai et al. 2011).

Given the role of PARP1 in initiating the response to various types of DNA lesions, increased PARP1 enzymatic activity suggests that affected cells may face persistent DNA damage. Indeed, elevated markers of DNA damage, including γH2AX and 53BP1 foci, have been detected in cellular, mouse, and human neuron models of SCA7 (Stoyas et al. 2020; Niss et al. 2021). A possible explanation for the accumulation of unresolved DNA breaks is that polyQ-ataxin-7 induces epigenetic dysregulation of DNA repair machinery (Fig. 1). Our own data from ChIP-Seq H3K9 acetylation profiling of cerebellum obtained from SCA7 266Q mice revealed widespread dysregulation of 33 genes involved in DNA repair (Switonski et al. 2021). Accumulation of DNA damage markers, DNA repair reporter assays, and genome-wide translocation profiling suggest that the canonical DNA repair interactome is functionally altered in SCA7 (Switonski et al. 2021). Indeed, excess production of reactive oxygen species (ROS) may contribute to the increased DNA damage detected in SCA7. Downregulation of key players in antioxidation defense, such as catalase and superoxide dismutase 2, together with induction of NADPH oxidase enzymes, was shown to increase ROS production in a PC12 model of SCA7 (Niss et al. 2021; Ajayi et al. 2012). This observation is supported by the correlation between oxidative stress and disease severity detected in SCA7 patients (Torres-Ramos et al. 2018). Interestingly, a mitochondrial origin for ROS accumulation could not be found in the SCA7 PC12 model (Ajayi et al. 2012). However, mitochondria are a major source of ROS in mammalian cells; consequently, their malfunction is commonly associated with ROS build-up (Cui et al. 2012). It is therefore plausible that mitochondrial dysfunction documented in SCA7 mice and SCA7 neurons derived from patient iPSCs contributes to increased ROS production and subsequent DNA damage accumulation in SCA7 (Fig. 1).

3 Targets for Therapeutic Interventions

3.1 Calcium-Activated Potassium Channels

Proper calcium homeostasis is fundamental for neuron function. Calcium ions are crucial in progressing an action potential, releasing neurotransmitters into the synaptic cleft, and as a secondary signaling messenger, in propagating information stored in a membrane potential to regulate cellular biochemical and metabolic processes (Gleichmann and Mattson 2011). Given the importance of calcium ions and their 10,000-fold concentration gradient across the neuronal membrane, it comes as no surprise that tight regulation of calcium cytosolic levels is crucial for neuron function and survival. Purkinje cells, with their autonomous pacemaker activity, exhibit high rates of spiking even in the absence of synaptic input, and are thus particularly sensitive to alteration in calcium buffering, transport, and storage (Bushart et al. 2016). Consequently, perturbations in calcium handling capacity have been found to be a central component of degeneration in numerous spinocerebellar ataxias, including SCA1, 2, 3, 6, 7, 14, and 15 (Robinson et al. 2020). As such, therapies aimed at correcting aberrant calcium homeostasis may alleviate progressive dysfunction and death of Purkinje neurons.

One of the mechanistic repercussions of altered calcium homeostasis in neurons is dysfunction of calcium-activated potassium channels. Two types of these channels—small-conductance calcium-activated K+ (SK) channels and large-conductance calcium-activated K+ (BK) channels—are activated by intracellular calcium ions that enter the cell following depolarization. SK and BK channels play a vital role in the repolarization and afterhyperpolarization of the membrane that occurs after action potential bursts. Both channels control intrinsic excitability of Purkinje cells by regulating the rate at which the membrane potential reaches the spike threshold (Edgerton and Reinhart 2003). A growing body of evidence suggests that BK channel dysfunction is mechanistically involved in various spinocerebellar ataxias. A recently discovered single loss-of-function mutation in the BK channel has been shown to produce mitochondrial defects and progressive cerebellar ataxia (Du et al. 2020). Similarly, genetic deletion of the BK channel in mice leads to impaired motor function and ataxic phenotypes (Chen et al. 2010; Sausbier et al. 2004). BK channel dysfunction has been described in mouse models of SCA1 and 2, where reduced BK channel expression results in the inability of Purkinje neurons to produce regular and frequent spiking events (Dell'Orco et al. 2015, 2017). In SCA1, viral-mediated expression of the BK channel improves motor phenotypes in ataxic mice and partially rescues Purkinje cell degeneration (Dell'Orco et al. 2015). Interestingly, pharmacological blockade of the BK channel with iberiotoxin combined with the negative modulation of the T-type calcium channel Ca_v3 subfamily produces irregular Purkinje cell spiking in the posterior cerebellum of control mice (Stoyas et al. 2020). This observation, which phenocopies electrophysiological changes observed in Purkinje cells of SCA7 mice, suggests that altered BK channel function is pivotal for SCA7 disease pathogenesis.

Overexpression of the BK channel in fxSCA7 92Q transgenic mouse Purkinje neurons via adeno-associated virus (AAV) stereotactic injection of BK channel cDNA into the deep cerebellar nucleus rescues Purkinje cell spiking irregularities (Stoyas et al. 2020). Thus, modulation of the BK channel is an appealing therapeutic approach for SCA7 and other spinocerebellar ataxias.

Similar to BK channel activation, stimulation of SK channels may offer a promising therapeutic strategy for SCA7, as it is hypothesized that pharmacological modulation of SK channels can compensate for reduced BK channel conductance (Bushart et al. 2018). The restoration of a proper Purkinje cell firing pattern through SK channel modulation has been demonstrated in several cerebellar pathologies. Episodic ataxia type-2 is caused by mutations in the $Ca_v2.1\alpha1$ subunit of the P/Q--type voltage-gated calcium channel that manifests in the loss of Purkinje cell firing precision (Jen et al. 2007). As such, episodic ataxia type-2 resembles SCA7, in which the diminished precision of Purkinje neurons is also associated with inadequate activation of calcium-activated potassium channels. Activation of SK channels with chlorzoxazone (a mixed SK/BK channel activator), 1-EBIO, and 4-aminopyridine in a mouse model of episodic ataxia type-2 restored precise firing of Purkinje cells and significantly improved motor performance (Alvina and Khodakhah 2010; Walter et al. 2006; Alviña and Khodakhah 2010). Combined treatment consisting of chlorzoxazone and the GABA-B agonist baclofen, which activates the subthreshold-activated potassium channel, rescued Purkinje cell spiking in Pcp-SCA1 82Q transgenic mice acute cerebellar slices and improved Purkinje cell electrophysiology and motor performance in SCA1 154Q knock-in mice (Bushart et al. 2018; 2021). Similar results were achieved by baclofen combined with SKA-31 or 1-EBIO, SK channel activators (Bushart et al. 2018). Treatment with SKA-31 has also proven to be beneficial in alleviating disease phenotypes in SCA3 YAC 84Q homozygous mice (Shakkottai et al. 2011). SCA2 is another example of a cerebellar degenerative ataxia where beneficial effects of SK channel modulation have been documented. In a series of studies, the Bezprozvanny laboratory tested a number of SK channel activators for their ability to normalize Purkinje cell activity and to rescue motor phenotypes in SCA2 model mice. NS309, a pan-SK channel modulator, corrected the firing pattern of aging Purkinje cells in SCA2 transgenic mice. Additionally, two related allosteric modulators of SK2/3 channels, CyPPA and NS13001, improved motor performance of SCA2 model mice and protected neurons from degeneration (Kasumu et al. 2012). Similarly, intraperitoneal injection of chlorzoxazone corrected the firing activity of SCA2 Purkinje neurons (Egorova et al. 2016).

Over the past few years, the SK channel modulation strategy has been clinically applied in SCA patients. Retrospective review of SCA1, 2, 6, 7, 8, and 13 patient charts from the University of Michigan Ataxia Clinic revealed that combined baclofen and chlorzoxazone treatment was tolerated in the majority of patients and improved symptoms according to the Scale for the Assessment and Rating of Ataxia (SARA) (Bushart et al. 2018). Two clinical trials to evaluate riluzole, a positive allosteric modulator of the SK2 channel, involved patients suffering from cerebellar ataxias of different etiologies. Both studies found that riluzole increases the

proportion of patients with an improvement in the International Cooperative Ataxia Rating Scale (ICARS) score (Ristori et al. 2010) or the SARA score (Romano et al. 2015) compared to the placebo group. The prospect of using CAD-1883 to relieve tremor and reduce ataxia and motor incoordination in SCA7 and other SCAs was recently proposed by Cadent Therapeutics. CAD-1883 is a positive allosteric modulator of the SK2/3 channel that renders the SK channel more sensitive to calcium ions. Previously, CAD-1883 successfully completed a Phase 2a clinical trial designed to evaluate its safety, tolerability, and efficacy in essential tremor patients (ClinicalTrials.gov Identifier: NCT03688685). The encouraging results from mouse model studies and initial clinical experience strongly support the idea that positive modulation of BK and/or SK channels could be an effective treatment for SCA7 and other forms of ataxia. Development of more potent and specific modulators, especially for the BK channel, could also diminish off-target effects caused by existing compounds and thereby improve their therapeutic potential.

3.2 SIRT1/NAD+ Pathway

Transcriptional alterations appear to be a common mechanistic component in polyQ diseases, including SCAs. Available data shows that transcriptional dysregulation emerges before the onset of disease symptoms, supporting the hypothesis that altered gene expression plays an early role in the neurodegenerative disease cascade (Helmlinger et al. 2006b). Transcriptional dysregulation is particularly relevant for SCA7 pathogenesis, in which the causative protein is a core component of the STAGA transcriptional coactivator complex. Consequently, global gene expression changes have been identified in cellular, mouse, and neuronal models of SCA7 (reviewed in (Niewiadomska-Cimicka et al. 2020)). Therefore, gene transactivation may be a reasonable therapeutic strategy to treat SCA7 and other polyQ disorders.

Disruption of the SIRT1/NAD+—PPARγ/PGC-1α regulatory axis has been implicated in the transcriptional repression of genes controlling calcium homeostasis in SCA7 (Fig. 1). Persistent NAD+ reduction in the cerebellum of SCA7 mice restrains the physiological activity of SIRT1 and consequently interferes with PGC-1α-dependent transcription (Stoyas et al. 2020). SIRT1 is one of the seven mammalian NAD+-dependent sirtuin deacetylases that act as sensors of metabolic conditions and adaptively respond via transcriptional modulation of numerous cellular processes (Grabowska et al. 2017). Many studies have documented the neuroprotective role of SIRT1 through its activation of mitochondrial biogenesis, mithophagy, glucose homeostasis, autophagy, and longevity-associated pathways (Xu et al. 2018).

3.2.1 SIRT1 Direct Activation

The therapeutic potential of SIRT1 activation in SCA7 has been confirmed by SIRT1 genetic overexpression of in two different SCA7 mouse models—fxSCA7 92Q transgenic mice and SCA7 266Q knock-in mice (Stoyas et al. 2020). SIRT1 overexpression at ~threefold endogenous levels improved motor function and delayed disease progression in both of these SCA7 mouse models. Moreover, SIRT1-overexpressing SCA7 266Q mice displayed significant amelioration of cerebellar degeneration, with improved Purkinje cell morphology and reduced gliosis (Stoyas et al. 2020). SIRT1 overexpression also improved survival of SCA7 266Q animals. Documented expression correction of 58 out of the 96 dysregulated genes suggests that SIRT1 executes its neuroprotective function via transcriptional derepression (Stoyas et al. 2020). Interestingly, SIRT1 overexpression and SIRT1 pathway activation have proven to be therapeutically effective in models of Parkinson Disease (PD), Alzheimer Disease (AD), Huntington's disease (HD), and SCAs (Watchon et al. 2021; Cunha-Santos et al. 2016; Bhalla et al. 2020; Cao et al. 2020; Jeong et al. 2011; Jiang et al. 2011; Wu et al. 2011; Chen et al. 2005; Kim et al. 2007). In some of these studies, the modulation of SIRT1 was achieved with resveratrol, a compound that allosterically activates sirtuins. Numerous clinical studies have subsequently validated safety and the beneficial effect on resveratrol in humans, including the AD study (reviewed in (Bonkowski and Sinclair 2016)). Specificity of resveratrol, however, is not limited to SIRT1 and it has been shown to interact with various other proteins, including AMPK, PARP1 and components of electron transport chain. Moreover, it displays variability in efficacy in clinical trials (Tomé-Carneiro et al. 2013). Thus, the prospect of finding specific sirtuin-activating compounds (STACs) is very attractive from a therapeutic perspective (Fig. 2). Some recently obtained STACs with improved bioavailability, including SRT1720, SRT3025 and SRT2014, yielded promising results in mice and were apparently on a path towards clinical trials (Bonkowski and Sinclair 2016). Whether they would be effective in SCA7 treatment is yet to be determined, but given the fact that the SIRT1 pathway deficiency can be rescued in SCA7 models with SIRT1 overexpression, allosteric SIRT1 modulation could be effective in alleviating disease symptoms.

3.2.2 NAD+ Replenishment

Another potential approach to activate the SIRT1 pathway is to increase NAD+ levels in affected cells. NAD+ is known to be generated through two processes: de novo biosynthesis and the salvage pathway. De novo synthesis utilizes dietary tryptophan and converts it into NAD+ in the multistep enzymatic process of the kynurenine pathway (Braidy et al. 2019). Most NAD+, however, is recycled from nicotinamide (NAM) and nicotinamide mononucleotide (NMN), the products of the NAD+ consumption reactions. In this process, salvaged NAM is first transformed into NMN by the nicotinamide phosphoribosyltransferase, which is subsequently adenylated into NAD+ by NMN adenylyltransferase (Xie et al. 2020). The salvage

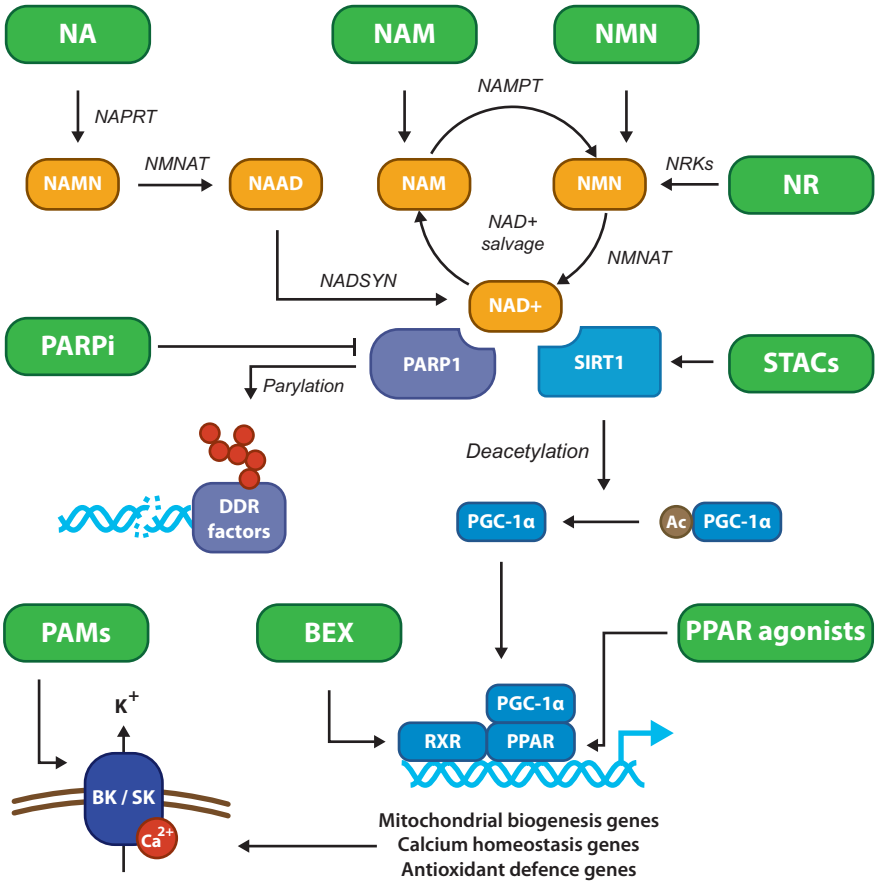

Fig. 2 Potential targets for SCA7 therapeutic intervention. The complex nature of the SCA7 pathogenic cascade offers the opportunity to therapeutically intervene at a variety of steps along disrupted pathways. NAD^+ salvage biosynthesis is a convenient gateway for NAD^+ boosting strategies that utilize NAD^+ precursors, which may be given as dietary supplements. To rebalance skewed PARP1/ SIRT1 NAD^+ consumption, either PARP1 inhibitors or SIRT1 activators are viable options to consider. Furthermore, small molecule agonists of RXR/PPAR nuclear receptors could restore the dysregulated transcription activation, which is observed in SCA7. Finally, as a consequence of disturbed calcium homeostasis, the function of calcium-activated potassium channels is altered in SCA7. Activation of these channels with positive allosteric modulators (PAMs) is currently being explored as a therapeutic strategy for SCA7 and other spinocerebellar ataxias

pathway is a convenient access point for potential NAD+ replenishment (Fig. 2). In one possible scenario, dietary supplemented nicotinamide riboside (NR) is phosphorylated into NMN by NR kinases. NR boosts NAD+ synthesis bypassing NAM–NMN conversion, the rate-limiting reaction in the salvage pathway. Beneficial aspects of NR supplementation have been documented in age-related pathologies (Elhassan et al. 2019). Moreover, NR clinical trials have been and are being

conducted for a variety of human disorders, including PD and AD (reviewed in (Rajman et al. 2018)). Sirtuin activation strategy mediated by NR-induced NAD+ replenishment has been preclinically tested on cellular and mouse models of SCA7. iPSC-derived human NPCs obtained from SCA7 patients showed a sustained increase in calcium concentration and increase in the variance of the calcium amplitude curve after potassium chloride-induced depolarization. NR treatment of SCA7 NPCs corrected both of these phenotypes, suggesting its neuroprotective potential (Stoyas et al. 2020). Similarly, NAD+ replenishment achieved in SCA7 266Q mice via NR dietary supplementation significantly ameliorated disease phenotypes and extended SCA7 mouse life span (Stoyas et al. 2020).

These promising results from the NAD+ replenishment approach suggest that other NAD+ boosting strategies could also be considered for SCA7 treatment. In addition to NR supplementation, other NAD+ precursors, including vitamin B3 (NAM, NA) and NMN also induce NAD+ repletion. Twelve-month-long NMN administration in the form of supplemented chow effectively mitigated age-associated physiological decline in wild-type mice. Consistent with the SCA7 study, NMN contributed to these anti-aging effects by reverting age-associated gene expression changes in peripheral tissues (Mills et al. 2016). NAD+ precursors have also shown therapeutic promise in alleviating phenotypes in other neurodegenerative diseases. Positive responses to NMN treatment have been documented in mouse models of AD (Wang et al. 2016; Long et al. 2015; Yao et al. 2017). NA supplementation improved the quality of life of PD patients (Alisky 2005; Chong et al. 2021), and neuroprotective effects of NAM with improvement in motor functions have been documented in an α-synuclein Drosophila model of PD (Jia et al. 2008). The downside of using NAD+ precursors as therapeutic agents is that they require large dosages to achieve a detectable effect, and one issue is the intellectual property landscape and lack of patentability, which may preclude their development by the biopharmaceutical industry. Further trials and development of novel NAD+ precursor compounds with better stability and enhanced NAD+ boosting capabilities, such as dihydronicotinamide riboside, could increase their therapeutic efficacy (Yang et al. 2019), and offer a path to the clinic.

3.3 PPAR:RXR:PGC-1α Pathway

Dysregulation of the PPAR/PGC-1α pathway induces transcriptional repression of numerous cellular processes, many of which have been mechanistically linked to SCA7 (Fig. 1). The PPAR family of ligand-activated transcription factors contains three subtypes: PPARα, PPARγ, and PPARδ (Dubois et al. 2017). Upon stimulation with endogenous ligands, including lipids and fatty acid derivatives, PPARs homodimerize or heterodimerize with the retinoic acid receptor (RXR) and then bind to PPAR response elements in their target genes (Tyagi et al. 2011). PPARs execute their transcriptional programs by recruiting a number of coactivator proteins, including PGC-1α.

Dubbed as a master regulator of mitochondrial biogenesis and energy expenditure, PGC-1α has been shown to control transcriptional programs responsible for fatty-acid β-oxidation, Krebs cycle, and oxidative phosphorylation (Puigserver and Spiegelman 2003). PGC-1α knock-out mice revealed that PGC-1α controls expression of a significant number of mitochondrial genes, and as such is crucial for proper mitochondrial translation, protein import, mitochondrial biogenesis and respiratory function (Lin et al. 2005). Moreover, PGC-1α stimulated the expression of ROS-detoxifying enzymes, including SOD1, SOD2, and catalase, and reduced mitochondrial ROS production by dissipating the proton gradient via stimulation of UCP2 and 3 uncouplers (St-Pierre et al. 2006; Lin et al. 2005).

3.3.1 PPARγ Activation

One attractive therapeutic strategy for SCA7 would be to elicit activation of PPAR/PGC-1α-dependent neuroprotective transcriptional programs (Fig. 2). Interestingly, PPARγ/PGC-1α has been established as a promising therapeutic target in Friedreich's ataxia (FRDA) which, analogous to SCA7, is characterized by increased oxidative stress, defective energy production, calcium dyshomeostasis and mitochondrial dysfunction (Schreiber et al. 2019). Cellular models of FRDA revealed that PPARγ activation with small molecule agonists, including pioglitazone, azelaoyl PAF, and leriglitazone, alleviate molecular phenotypes associated with the disease (Marmolino et al. 2009, 2010; Rodríguez-Pascau et al. 2021). Leriglitazone also improved motor deficits and ataxia in FRDA model mice (Rodríguez-Pascau et al. 2021) and recently showed clinical benefit in 32 FRDA patients in a Phase 2 randomized, placebo-controlled study (ClinicalTrials.gov Identifier: NCT03917225).

3.3.2 PPARδ Activation

Yet another strategy to induce neuroprotective effects of the PPAR/PGC-1α pathway is to allosterically target PPARδ. Among all PPAR family members, PPARδ is highly expressed in skeletal muscle and is the most abundant PPAR subtype in the brain. Similar to PPARγ, PPARδ cooperates with PGC-1α at executing transcriptional programs responsible for energy metabolism, mitochondrial biogenesis, inflammatory processes, and ROS defense (Strosznajder et al. 2021). Expression of dominant-negative PPARδ in mouse central nervous system (CNS) produces molecular, histopathology, and motor phenotypes resembling neurodegenerative conditions, thus demonstrating the important role of PPARδ in neuropathological processes (Dickey et al. 2016). Although the functional relevance of PPARδ in CNS is still being actively studied, it is becoming increasingly clear that allosteric activation of PPARδ can produce numerous beneficial effects in neurodegenerative diseases. The PPARδ agonist GW0742 has been extensively tested in preclinical studies using rodent models of AD, PD, and CNS hypoxia/Ischemia. Orally administered GW742 reduced amyloid plaque formation and yielded a prominent

anti-inflammatory effect by reducing astrocyte and microglial activation in 5xFAD AD mice (Malm et al. 2015; Kalinin et al. 2009). Moreover, GW742 significantly improved learning deficits in mice with $A\beta_{1-42}$ aggregated oligomers infused into the hippocampus (An et al. 2016). In the MPTP rat model of PD, GW742 resulted in significant improvement in cognitive impairment on the Morris water maze test, ameliorated oxidative damage and DNA fragmentation, and rescued tyrosine hydroxylase reduction in TH-positive neurons (Das et al. 2014). Similar neuroprotective effects of PPARδ activation, induced by yet another PPARδ agonist GW501516, have been observed in the MPTP mouse model of PD (Chen et al. 2019; Iwashita et al. 2007). We have documented that KD3010, a potent PPARδ agonist initially developed as a potential type 2 diabetes drug, achieves dramatic protection against neuronal dysfunction in HD model mice, as intraperitoneal injection of KD3010 alleviated HD-related neurological dysfunction, improved motor coordination, and markedly extended lifespan in N171-82Q transgenic HD mice (Dickey et al. 2016). Furthermore, striatal medium spiny-like neurons differentiated from HD patient iPSCs displayed significantly increased resistance to cell death when treated with KD3010 (Dickey et al. 2016).

3.3.3 RXR Activation

Another PPAR activation strategy that could promote PPAR/PGC-1α neuroprotective pathways is to target RXR (Fig. 2). Agonizing RXR, which forms heterodimers with activated PPARs, can boost PPAR transactivation of their target genes (Evans and Mangelsdorf 2014). Bexarotene, a selective RXR agonist, has yielded beneficial effects in several models of neurodegenerative diseases, including AD, PD, ALS, and epilepsy, although the neuroprotective effects achieved in AD remain controversial (Vidal et al. 2021). We have found that bexarotene is neuroprotective in a number of cellular models of HD, including mouse striatal and cortical neurons and human medium spiny-like neurons derived from HD patient iPSCs (Dickey et al. 2017). We also completed a preclinical therapy trial of bexarotene in N171-82Q HD transgenic mice, and we found that bexarotene treatment prevented neuron loss in the striatum and improved motor performance and survival. Our findings indicate that bexarotene-mediated PPAR activation can improve mitochondrial and protein quality control pathways in HD (Dickey et al. 2017). Mechanistically, SCA7 is characterized by mitochondrial dysfunction, increased ROS burden, and dysregulated calcium handling capacity arising from disrupted expression of the PPAR/PGC-1α network genes. This fact, together with the encouraging results obtained in preclinical trials of PPAR/RXR activators in other neurodegenerative diseases, suggests that pharmacological intervention with PPAR:RXR agonists deserves serious consideration as a potential therapy for SCA7.

3.4 DNA Damage

Sustained DNA damage occurring in SCA7 is believed to be fueled by increased oxidative stress and an epigenetically dysregulated DNA repair response (Fig. 1). As a result, PARP1 hyperactivation depletes NAD+ levels, leading to SIRT1-dependent transcription dysregulation (Stoyas et al. 2020). Modulation of the anti-oxidative stress response via activation of the PPAR/PGC-1α regulatory pathway could potentially alleviate DNA damage accumulation and counter excessive PARP1 engagement. A strategy of boosting NAD+ levels, agonizing SIRT1, and activating PPAR:RXR factors, has just been described. Another viable approach to counteract PARP1 hyperactivation is to directly target PARP1 with a specific PARP1 inhibitor (PARPi). Many PARPis have already been approved as a treatment for cancers of the breast, ovary, lung, and pancreas. Their mechanism of action has been a topic of extensive research and has led to a model whereby homologous recombination (HR)-deficient tumors cannot properly repair PARPi-induced collapse of replication forks (Rose et al. 2020). PARPis have also been shown to improve metabolic homeostasis by increasing NAD+ levels and SIRT1 activity (Zha et al. 2018). Although modulation of PARP1 has mainly been researched as a cancer treatment, growing evidence suggests that PARP1 inhibition may represent an attractive therapeutic strategy for neurodegeneration. The reason that PARP1 has attracted notable attention in the neurological disease field is the pivotal role that PARP1 plays in regulation of oxidative stress-induced inflammation and microglial activation. As chronic neuroinflammation is a common theme across numerous CNS pathologies, altered PARP1 activation has been implicated in PD, AD, HD, ALS, retinal degeneration, and other neurodegenerative disorders (Pazzaglia and Pioli 2019). In addition to neuroinflammation, other PARP1-regulated processes, including autophagy and apoptosis, have been mechanistically linked to neurodegeneration (Mao and Zhang 2021).

Olaparib, a potent PARP1 and 2 inhibitor, induces in vitro and in vivo neuroprotection in a mouse model of hereditary retinal degeneration (Sahaboglu et al. 2016). Similarly, olaparib treatment demonstrated neuroprotective effects and reduced neuron death in an NPC model of Schinzel-Giedion syndrome (Banfi et al. 2021). However, olaparib cannot cross the blood-brain barrier; thus, its therapeutic potential in neurodegeneration treatment is significantly restricted. Other PARPis with increased brain penetration are likely to be better suited for in vivo application. IP injected INO-1001 suppressed neuron loss and microglial activation and extended survival in the R6/2 mouse model of HD (Cardinale et al. 2015). Microglial activation is also attenuated in the hAPPJ20 AD mice upon PJ34 treatment (Kauppinen et al. 2011). Veliparib is a small molecule PARPi that inhibits PARP1 and 2 at nanomolar concentrations. Veliparib crosses the blood-brain barrier and shows good bioavailability. Although not yet approved for clinical practice, veliparib has demonstrated promising performance in preclinical cancer trials (Boussios et al. 2020). Recent studies indicate that veliparib is a potent neuroprotective PARP modulator in numerous CNS pathologies. Oral administration of veliparib promoted

autophagy flux, reduced neurotoxicity, and improved motor performance in the α-synuclein A53T mouse model of PD (Mao et al. 2020). Similarly, veliparib protected against excessive neuron cell death after brain ischemic injury in ADP-ribose-acceptor hydrolase 3-deficient mice (Mashimo et al. 2019). Moreover, a recent ALS study found that veliparib reduces the formation of stress-induced aggregates of TDP-43 in mammalian cells and inhibits TDP-43-associated neuronal death in primary rat spinal cord cultures (McGurk et al. 2018). Collectively, encouraging results from PARP1 inhibition in neurodegenerative disorders advocate for the design of future PARPi-based trials for SCA7 and other diseases where PARP1 hyperactivation disrupts cellular homeostasis (Fig. 2). However, important caveats should be taken into consideration. Cancer therapies, which introduced PARPis into the clinic, and neurodegeneration therapies have a fundamentally opposite desired outcome. While the goal of cancer treatment is to promote cell death, the goal of neurodegenerative disease therapy is to promote cell survival. This apparent paradox of achieving the two opposite objectives with a single class of therapeutic molecules illustrates the potential risk of the PARPi approach. Correcting excessive PARP1 engagement to restore neural health without any deleterious effects due to inhibition of PARP1 activity may not be feasible. It may be even more complicated in SCA7, where the available data suggest that dysregulation of the HR repair pathway contributes to neuronal dysfunction (Switonski et al. 2021). Hence, while PARPis are most toxic to HR-deficient tumor cells, any PARPi-based SCA7 therapy would require fine tuning to prevent detrimental side effects stemming from too much inhibition of PARP activity in neurons and other CNS cell types.

3.5 Reducing Ataxin-7 Expression

As a monogenic, dominantly inherited disease, SCA7 is an ideal candidate for any treatment that can prevent the expression of the CAG-expanded *ATXN7* gene. Several technologies targeting mRNAs of *ATXN7* and other polyglutamine disease genes are currently being developed. Because the goal of therapeutic dosage reduction is to block mutant protein production, any pathology cascading down from the polyQ gain-of-function mechanism should be interrupted, underscoring the huge potential for a powerful therapeutic effect from this strategy.

3.5.1 RNAi Effectors

The RNA interference (RNAi) pathway, which endogenously regulates gene expression in a sequence-dependent manner, has been pursued in basic research and therapeutic trials for over two decades. RNAi is triggered by the presence of small interfering RNAs (siRNA) or microRNAs (miRNA), 21–23 nucleotide long non-coding RNA molecules that are incorporated into the RNA-induced silencing complex (RISC). The siRNA- or miRNA-loaded RISC binds to the target mRNA and

either initiates mRNA cleavage (siRNA) or mRNA deadenylation, degradation, or translation inhibition (miRNA) (Setten et al. 2019) (Fig. 3). The first preclinical attempts using RNAi to silence mutant genes responsible for neurodegenerative diseases were made in the mid-2000s in SCA1, HD, and SCA3 mouse models. All of these studies reported successful silencing of the mutant allele and alleviation of neurodegenerative and motor phenotypes (reviewed in (Afonso-Reis et al. 2021)).

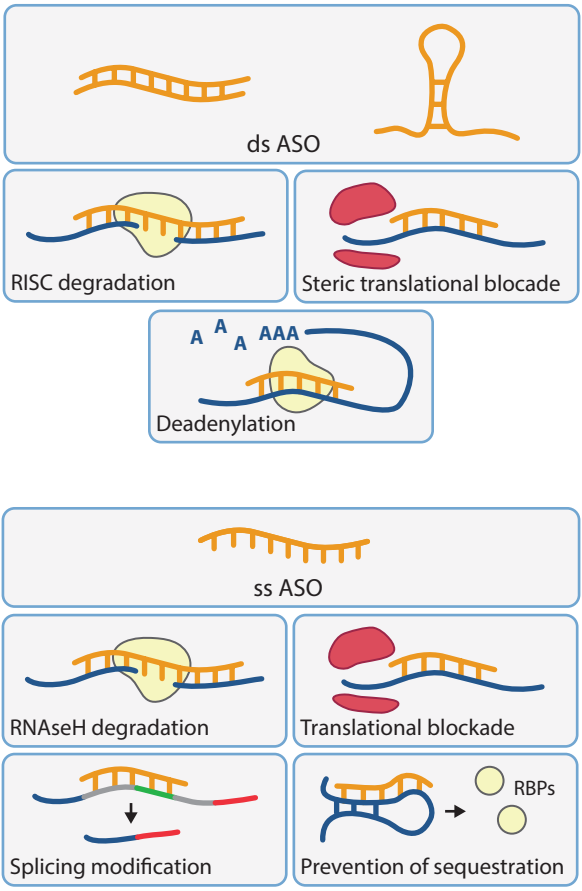

Fig. 3 Molecular mechanisms of antisense oligonucleotides (ASOs). An RNAi effector in the form of either a RNA duplex, shRNA or miRNA precursor will hybridize with the target mRNA and initiate RISC-mediated mRNA cleavage (siRNA) resulting in transcript degradation, RISC-mediated mRNA deadenylation, or steric translational inhibition (miRNA). The primary mode of action for an *ATXN7*-targeting single-stranded DNA ASO is mRNA degradation that occurs when RNAase H1 hydrolyzes RNA/DNA hybrids. ASO-mediated steric translational blockage is yet another mechanism that may lead to the reduction of ataxin-7 protein expression. Additionally, other mechanistic scenarios achieving different molecular effects (including splicing modulation and/or release of sequestered RNA binding proteins [RBPs]) have been validated for ASOs in other repeat diseases

Non-allele selective RNAi-based approach has been evaluated in SCA7 transgenic mice by targeting both human and mouse *ATXN7* genes. Injection of the AAV vector encoding artificial miRNA named miS4 into deep cerebellar nuclei reduced ataxin-7 expression to about 50% (Ramachandran et al. 2014b). Mice injected at 7 weeks of age were followed until 40 weeks of age and evaluated for histopathological anomalies and motor performance. Rotarod and gait analysis showed that miS4 injection significantly alleviated motor phenotypes in SCA7 mice. Moreover, RNAi-mediated ataxin-7 silencing improved progressive SCA7 neuroinflammation phenotypes and rescued other molecular phenotypes, such as nuclear inclusion formation and dysregulated gene expression (Ramachandran et al. 2014b). The feasibility of a non-allele selective approach using miS4 has been also investigated in the retina of fxSCA7 92Q transgenic mice, despite lack of a severe retinal phenotype observed in this genetic model. Importantly, ataxin-7 knockdown does not interfere with normal retinal function and shows no apparent toxicity even 23 weeks after subretinal injection of the miS4 AAV vector (Ramachandran et al. 2014a). In vivo evaluation of the non-allele selective *ATXN7* silencing strategy addresses crucial safety concerns regarding inadvertent suppression of ataxin-7 normal function. Previous studies have shown that significant reduction of normal ataxin-7 levels in zebrafish and fruit fly leads to impaired differentiation of photoreceptors in the retina and in Purkinje and granule cell neurons in the cerebellum (Yanicostas et al. 2012; Carrillo-Rosas et al. 2019). It is worth noting that both miS4 studies reported ~50% silencing efficiency of ataxin-7, implying retention of at least 25% of normal ataxin-7 function.

The *ATXN7* locus is located on an autosome (chromosome 3); hence, a typical SCA7 patient is heterozygous, with one normal allele and one CAG expanded allele (Benomar et al. 1995; Gouw et al. 1995). The most straightforward silencing strategy is to utilize an oligonucleotide that targets both normal and mutant alleles and leads to global reduction in *ATXN7* gene expression (Fig. 4). The attractiveness of this approach is that there are fever constraints in choosing the target sequence. Consequently, there are more possibilities of identifying an effective ASO that could be applicable to the whole population of SCA7 patients. Alternatively, to avoid potential repercussions of knocking down normal ataxin-7 function, an allele-unique oligonucleotide target sequence could be chosen (Fig. 4). Single-nucleotide polymorphisms (SNP) associated with CAG expansions, which can be used to discriminate between two alleles, have been identified (https://www.ncbi.nlm.nih.gov/snp/?term=atxn7). Unfortunately, not a single SNP covers all SCA7 patients. The strongest association between SNP and the CAG mutation has been identified in the South African population. A founder effect is responsible for this phenomenon, where the A variant of the SNP rs3774729 coincides with 100% of CAG expanded alleles, with 43% of the South African SCA7 patients being heterozygous in the rs3774729 locus (Greenberg et al. 2006). An alternative approach that could potentially overcome the lack of full coverage of the SCA7 patients population is to discriminate between alleles using the differences in the CAG repeat length (Fig. 4). It has been demonstrated that RISC loaded with artificial miRNA directed against the CAG region binds to more target sites on the expanded allele and cooperatively

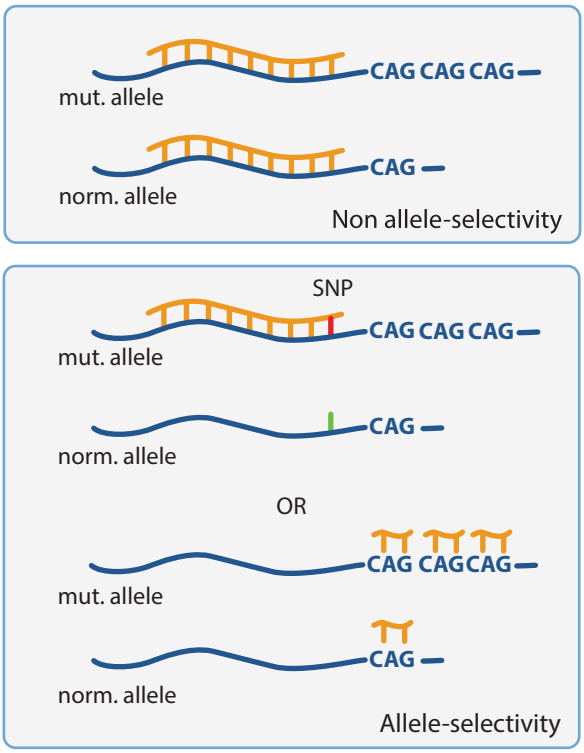

Fig. 4 Non-allele selective vs. allele selective ASO-mediated silencing of *ATXN7* mRNA expression. Targeting the sequence common for both normal and mutant allele leads to global reduction of *ATXN7* gene expression (TOP). Such a non-allele selective design offers more options for selecting the ASO sequence, but also targets the normal allele; consequently, reduced expression of normal protein may limit the therapeutic success of this silencing strategy. The alternative strategy, though technically much more challenging, is to achieve allele selectivity (BOTTOM) by either (i) targeting a disease allele-linked single nucleotide polymorphism (SNP) OR (ii) directly targeting the expanded CAG stretch itself. In the latter case, the longer CAG sequence present on the mutant allele permits a greater number of CAG-targeting ASOs to bind, resulting in cooperative selective repression of mutant allele expression

represses its expression via deadenylation and translation inhibition (Ciesiolka et al. 2021). The trials on dermal fibroblasts from SCA7 patients revealed the therapeutic potential of allele-selective RNAi effectors like SiR-P16. SiR-P16 is a potent siRNA that uses SNP rs377472 to differentiate between mutant and normal ataxin-7 transcripts. This siRNA was identified by screening a large number of short hairpin RNAs (shRNAs) (Scholefield et al. 2009). SiR-P16 efficacy was subsequently tested in SCA7 fibroblasts, where it induces selective knockdown of the mutant allele and corrects the expression of two genes altered in SCA7 fibroblasts, *DNAJA1* and *UCHL1* (Scholefield et al. 2014). Similarly, CAG repeat-targeting RNAi reagents that form mismatches with the target region and consequently block mRNA translation have been tested in SCA7 fibroblasts. Such a strategy results in effective

and selective knockdown of polyQ-ataxin-7 that is accompanied by upregulation of the *UCHL1* gene (Fiszer et al. 2016a, b).

One interesting aspect of developing CAG repeat-targeting reagents is that they can be potentially adapted for the treatment of other polyQ diseases. The proof of concept of such an approach has been successfully demonstrated in SCA3, SCA7, DRPLA, and HD models by both Corey and Krzyzosiak laboratories (Fiszer et al. 2013, 2016a; Gagnon et al. 2010; Hu et al. 2010). Recently, a CAG repeat-targeting reagent in a form of lentivirus-delivered shRNA has shown selective transcriptional repression of mutant *HTT*, *ATN1*, *ATXN3*, and *ATXN7* genes in HD, DRPLA, SCA3, and SCA7 patient fibroblasts, respectively. Importantly, analysis of unrelated genes containing between 13 and 33 CAG repeat regions has shown no significant off-target effect of shRNA treatment (Kotowska-Zimmer et al. 2020). Yet another option that leads to suppression of mutant causative genes but preserves their normal counterpart is to non-selectively knock down both alleles and concurrently reintroduce a normal variant. Such a knockdown-replacement strategy employing ataxin-7-targeting artificial precursors of miRNA (mirtron) and expression of mitron-resistant ataxin-7 functional copy were recently validated in SCA7 patient fibroblasts (Curtis et al. 2017). Admittedly, an ideal drug development strategy seeking to silence *ATXN7* gene would seek the ideal balance between therapeutic benefits and toxicity induced by the theoretical detrimental effect of ataxin-7 loss of function; however, as ataxin-7 is part of a multigene family and no adverse effects occur upon knock-out of ataxin-7 in mice, it is likely that partial loss of ataxin-7 function in human patients subjected to non-allele specific dosage reduction therapy will be well tolerated.

3.5.2 Antisense Oligonucleotides (ASOs)

Upon entering a cell, ASOs bind to their target mRNA molecules in a sequence-specific manner. Depending on the ASO class, an oligonucleotide/mRNA hybrid induces mRNA degradation, modulates splicing, or serves as a steric blockade for translation (Fiszer and Krzyzosiak 2014). The ability of a single-stranded ASO to modulate gene expression was demonstrated over 40 years ago when Zamecnik and Stephenson documented oligonucleotide-mediated inhibition of viral replication in a chicken embryo (Zamecnik and Stephenson 1978). Since that initial discovery, decades of research have focused on optimizing ASO chemistry, solving its mechanism of action and finally transferring the technology from bench to bedside. One of the first obstacles that researchers encountered was poor stability of single-stranded DNA and RNA molecules that are readily degraded by cellular and extracellular nucleases. To overcome this issue and to improve ASO binding-affinity, chemical modifications have been introduced to the ASO phosphate backbone and ribose, including, but not limited to, phosphorothioates (PS), 2′-O-methyl (2′-OME), and 2′-O-methoxyethyl (2′-MOE) nucleotides. Introduction of new DNA/RNA analogs, such as peptide nucleic acids (PNAs), morpholino, locked nucleic acids (LNAs), constrained methoxyethyl (cMOE) and constrained ethyl (cEt)

nucleoside analogs further increased in vivo potency, cellular uptake, and pharmacokinetic profiles of ASOs (Crooke et al. 2021). This myriad of available chemical modifications illustrates the fact that ASOs are very adaptable molecules with a huge potential for further optimization of their therapeutic properties. ASOs can be customized based on their sequence, chemistry, and even chirality of modified phosphate backbone linkages (Silva et al. 2020). Current ASO-based therapeutic approaches take advantage of the versatile nature of these molecular agents to modulate their efficacy, retention time, toxicity profile, and to achieve the desired mechanism of action.

Similar to the RNAi effectors, single-stranded ASOs exert their gene silencing effects through a variety of molecular mechanisms that depend on ASO chemistry and a sequence context within the target mRNA molecule (Quemener et al. 2020) (Fig. 3). Degradation of the mRNA in the ASO/mRNA duplex is a highly desirable outcome in the therapy of SCA7 and other diseases where the pathology cascades down from the expression of a single mutant gene. DNA-based ASOs have been shown to recruit RNaseH, a ubiquitously expressed non-sequence-specific nuclease that catalyzes the cleavage of RNA in the DNA/RNA heteroduplex (Wu et al. 2004). Certain ASO chemistry, including PS modification, promotes RNaseH activation and facilitates mRNA breakdown, thus reducing unwanted protein expression. New generations of ASOs, however, with the modified 2′ ribose position, such as 2′-OME, 2′-MOE, LNA, and cEt, are unable to activate RNaseH. Their mechanism of reducing gene expression is believed to be linked to steric translational blockade (Quemener et al. 2020). In order to capitalize on both the benefits of potent modifications and RNAseH-mediated mRNA degradation, a gapmer approach has been conceptualized. Gapmer refers to the sequence of at least five unmodified or PS-modified nucleotides activating mRNA cleavage, flanked by non-RNaseH-activating parts of an ASO (Monia et al. 1993).

It is important to highlight that the molecular effects achieved with a single-stranded ASO could reach far beyond the suppression of gene expression (Fig. 3). ASOs that do not elicit RNaseH recruitment, designed to target specific exon-intron junctions, are instead used to eliminate aberrant splicing arrangement. Such a strategy can be also used to exclude a mutation-bearing exon from the mature mRNA. The strategy to use ASOs as splicing modulators has been successfully validated in both preclinical and clinical trials (Havens and Hastings 2016). Additionally, ASOs have been adapted to release proteins abnormally sequestrated by anomalous RNA structures. Myotonic dystrophy (DM1) is a dominant neuromuscular disease caused by the CUG expansion in DM protein kinase (DMPK) mRNA. Mutant transcripts sequester alternative splicing factors, including muscleblind-like 1 (MBNL1). Morpholino ASOs targeting expanded CUG triplets competitively release MBNL1 from its abnormal interaction and rescue aberrant splicing in DM1 mice (Wheeler et al. 2012). A similar approach has been recently demonstrated for fragile X-associated tremor/ataxia syndrome (FXTAS), where toxic sequestration of proteins involved in RNA metabolism initiates a pathogenic cascade resulting in tremor and cerebellar gait ataxia. ASO steric blockade released RNA binding proteins sequestered by expanded CGG repeats, improved motor performance, and corrected

molecular phenotypes in the FXTAS mouse model (Derbis et al. 2021). Several other therapeutic applications of single-stranded ASOs, such as modulation of miRNA machinery (via masking miRNA sites or sequestering pathological miRNAs), increasing protein levels (via redirecting of translational operations), and modulation of mRNA maturation have been successfully pursued in preclinical trials (Quemener et al. 2020).

Since 1998, when the first single-stranded ASO (fomivirsen) was authorized by the FDA for cytomegalovirus retinitis treatment, antisense technology emerged as a robust platform to treat a wide variety of neurological, metabolic, cardiovascular, and muscular genetic conditions (Scharner and Aznarez 2021). Similar to RNAi, the therapeutic potential of single-stranded ASOs have been extensively studied in SCA7 and other polyglutamine repeat diseases. Preclinical studies using HD mouse and primate models have demonstrated beneficial effects of both non-allele and allele selective ASO-mediated huntingtin silencing as well as exon skipping strategy removing toxic caspase cleavage sites from the huntingtin protein (Kordasiewicz et al. 2012; Stanek et al. 2013; Casaca-Carreira et al. 2016; Southwell et al. 2014, 2018; Rué et al. 2016; Sun et al. 2014; Datson et al. 2017). In both SCA1 and SCA2 mouse models, ICV injection of the RNaseH-activating ASO significantly reduces mRNA levels of ataxin-1 and 2, respectively, and rescues disease associated phenotypes (Friedrich et al. 2018; Scoles et al. 2017). An exon skipping strategy aimed at removing exon 10 containing CAG repeats from mature *ATXN3* mRNA has been examined in SCA3 patient fibroblasts and mouse models. These studies delivered promising initial results regarding alternative splicing kinetics, ASO toxicity, and the ability to reverse molecular phenotypes associated with protein aggregation (Evers et al. 2013; Toonen et al. 2017). In recent work from the Paulson and McLoughlin groups, a screen of non-allele selective ASOs that elicit degradation of the *ATXN3* transcript has delivered therapeutic candidates showing broad distribution, low toxicity and potent silencing efficacy in SCA3 mice (Moore et al. 2017). Longitudinal preclinical therapy with the selected ASO rescued slowed Purkinje cell firing frequency, improved motor performance and reduced protein accumulation phenotypes in SCA3 YAC Q84 homozygous animals (McLoughlin et al. 2018). SCA1 and SCA3 genetic mouse models have also been used to assess efficacy of the ASO strategy targeting the elongated CAG region. Weekly ICV infusions of the 2′-OME-PS-modified CUG7 resulted in significant reduction of mutant ataxin-1 and 3 proteins throughout the mouse brain tissue (Kourkouta et al. 2019).

We have recently evaluated ASO treatment for SCA7 retinal cone-rod dystrophy in SCA7 266Q knock-in mice (Fig. 5). An ASO composed of cET nucleoside analogs targeting *ATXN7* mRNA was selected for high potency and low toxicity in a mouse endothelial cell line screen (Niu et al. 2018). Intravitreal injection (IVI) of the ASO resulted in substantial diffusion throughout the eye and effective delivery to the retinal photoreceptors. Six weeks post-IVI, retinal expression of ataxin-7 mRNA was reduced by more than 60% compared to vehicle-treated retinas. Consequently, aggregation of polyQ-ataxin-7 into insoluble nuclear inclusions was markedly reduced in retinal cells, including photoreceptors (Niu et al. 2018). ASO treatment also ameliorated other well-characterized SCA7 retinal disease

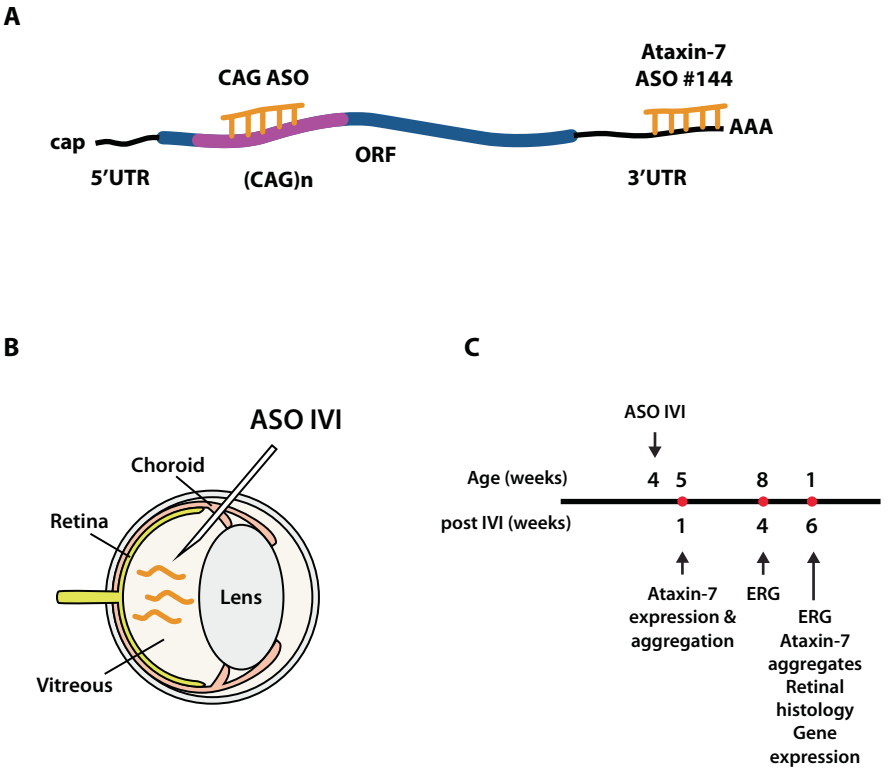

Fig. 5 Overview of the ASO preclinical trial for the treatment of retinal degeneration in SCA7 mice. (**a**) Diagram showing the two different ASOs tested in SCA7 preclinical trial: a CAG-specific ASO and a non-allele selective ASO directed against the mouse ataxin-7 mRNA in its 3' UTR. (**b**) Delivery of ASO was achieved by intravitreal injection (IVI). (**c**) Organizational design of the preclinical prevention trial. Note that the therapeutic ASO was delivered to one eye at 4 weeks of age, while PBS or a scrambled ASO was delivered to the opposite eye in the same individual mouse. Read-outs for the preclinical trial are listed with their respective time points after IVI delivery

phenotypes. Thinning of three retinal layers, the inner and outer segments of rods and cones, the outer nuclear layer (ONL) and the inner plexiform layer (IPL), was reduced in *ATXN7* ASO injected eyes. Moreover, the *ATXN7* ASO prevented dysregulated gene expression and blunted impaired epigenetic regulation of rhodopsin, m-opsin, and s-opsin (Niu et al. 2018). Importantly, the encouraging amelioration of molecular and histopathological deficits was accompanied by a significant improvement in visual function in *ATXN7* ASO-treated SCA7 266Q knock-in mice. Electroretinogram (ERG) analysis revealed that the *ATXN7* ASO enhanced both cone and rod photoreceptor function at both 4 weeks and 6 weeks post-IVI. We also documented similar, yet slightly less robust therapeutic outcomes using the ASO targeting elongated CAG repeat sequence of ataxin-7 mRNA. Upon IVI, CAG targeting ASO selectively silenced polyQ ataxin-7 and successfully rescued molecular,

histopathological, and visual phenotypes up to 4 weeks after injection. However, at 6 weeks post-IVI, therapeutic enhancement of cone and rod function was dominated by the disease progression (Niu et al. 2018). It is important to emphasize that all of this preclinical work was performed with careful attention to guidelines for rigor and reproducibility (Landis et al. 2012; Perrin 2014), by randomly assigning mice to groups, balancing for weight, gender, and baseline appearance (i.e., we excluded outliers on baseline behavioral studies) and by setting cohort sizes based on power analysis of read-outs. Good experimental practice also requires that all behavioral analysis and scoring of histopathology results be performed by researcher team members blinded to genotype and treatment status.

Promising results reported in cellular, mouse, and primate model studies have paved the way for clinical investigations of ASO therapeutic potential in polyQ diseases. Several companies have recently launched clinical trials for ASO-based HD treatment. At the beginning of 2021, five ASO drug candidates were investigated in Phase 1 and 2 human studies and one, Tominersen, in a Phase 3 trial involving nearly 800 patients. There are still, however, underlying uncertainties regarding ASO target engagement, efficacy, and mechanism of adverse effects in human trials. While Tominersen successfully progressed through Phase 1 and 2a clinical trials showing dose-dependent reductions in mutant huntingtin and no serious adverse effects (Tabrizi et al. 2019), Roche halted the Phase 3 trial after a review of the ongoing data revealed that the treatment group was performing worse than the placebo group on the Composite Unified Huntington Disease Rating Scale (cUHDRS) and Total Functional Capacity, as well as on other measures. Similarly, Wave Therapeutics' two allele-selective ASO Phase 1 and 2 trials were suspended after neither drug treatment showed the expected efficacy in silencing huntingtin gene (Kingwell 2021). Factors including dosing strategy, delivery route (both influencing penetration of a drug into deep cerebral structures), disease progression status at treatment initiation, and the lack of selectivity of the ASO towards polyQ-expanded huntingtin may have moved the outcome in the wrong direction. Continued thorough inspection of the clinical trial data is ongoing, as an understanding of the reason(s) for the failed Phase 3 trial will be critical for future therapeutic designs and clinical trials being planned for SCA7 and related disorders.

Indeed, a refined understanding of ASO dosage, route of delivery, target engagement, off-target activity, and non-allele specific toxicities will be needed to successfully develop ASO treatments for SCA7 and neurodegenerative diseases.

4 Concluding Remarks

Like all other dominant, gain-of-function neurodegenerative diseases, SCA7 is currently incurable. Decades of intensive research, however, including recent advances in a mechanistic understanding of SCA7 etiology and progression have uncovered many attractive targets that are bringing us closer to novel symptomatic and preventive treatments. This encouraging surge of therapeutic options still requires further

preclinical and clinical validation, while we concomitantly pursue other novel promising options. In particular, deciphering the basis for the selective vulnerability of Purkinje cells and retinal photoreceptors, upon which we have focused our research efforts for more than two decades, should reveal treatment strategies aimed at better protecting vulnerable neurons. This is an important and critical challenge, as most SCA7 patients will receive treatment after neuron dysfunction and degeneration is well underway. Furthermore, the complex SCA7 molecular cascade, with many different factors contributing to disease development, suggests that there should be multiple drug targets; hence, we envision the development of multiple drugs against various targets, and expect that SCA7 patients will be managed with a paradigm of combination therapy consisting of as many as three or more drugs. We remain especially enthusiastic for clinical deployment of ASOs targeting the ataxin-7 mRNA, as this therapeutic strategy is close to clinical trial entry and may yield compounds that could potentially halt progression of the disease in affected individuals and perhaps even prevent disease onset in presymptomatic patients.

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Experimental Neurotransplantation for Cerebellar Ataxias

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Abstract Neurotransplantation is one of the therapeutic methods for cerebellar diseases that are currently being intensively investigated. Despite years of research, there are still many questions to be answered. Compared to transplantation of other tissues and organs, neurotransplantation has some specific challenges, and these are particularly demanding for the cerebellum. In this chapter, the goals of neurotransplantation, its mechanisms underlying graft effects, the types of grafts, as well as the problems of graft survival, differentiation, and functional integration are discussed. Different requirements and limitations of neurotransplantation therapy related to different types of pathologies are also discussed. Finally, overview of neurotransplantation research employing animal models of diseases during past decades is provided.

Keywords Cerebellar ataxia · Grafting · Neurotransplantation · Purkinje cells · Stem cells

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_18

Abbreviations

BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
EVs	Extracellular vesicles
GDNF	Glial-derived neurotrophic factor
IGF-1	Insulin-like growth factor 1
KI	Knock-in
miRNA	microRNA
MSCs	Mesenchymal stem cells
NSCs	Neural stem cells
SCA	Spinocerebellar ataxia

1 Introduction

Transplantation or grafting means the transfer of organs, tissues, or cells from one position to another. In the case of autologous transplantation, donor and host are the same individual and there are no problems with immune incompatibility. Syngeneic transplantation is transfer between individuals who are genetically identical and thus immunologically identical. The most frequent type of transplantation is allogenic, where tissue transfer is between genetically and immunologically different individuals of the same species. Finally, xenografts come from another species. Neurotransplantation is grafting neural tissue or neural or stem cells to treat nervous system diseases or dysfunctions. It is considered a promising approach to treat neurological diseases. Nevertheless, it has not become a routine method and, for the cerebellum, it remains controversial, being still at the stage of animal studies despite tens of years intensive investigation (Rossi and Cattaneo 2002). Here we review specific features of cerebellar transplantation: graft types and potential mechanisms of their effects, factors playing roles in graft development and integration, and factors determining the potential benefits and limitations of neurotransplantation therapy in different diseases.

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2 Specific Features of Neurotransplantation and Cerebellar Transplantation

Compared to transplantations in other tissues, neurotransplantation has several specific features because the nervous system and its diseases also show specific aspects. Most of these features make neurotransplantation difficult and problematic.

First, the nervous system, particularly the central nervous system (CNS) has a relatively low regenerative capacity. Massive neuronal loss cannot be simply repaired just by neurogenesis and fiber restoration. Therefore, delivery of neurons or cells that can differentiate into neurons could be important to support functional recovery in CNS disease. On the other hand, neural plasticity could alleviate quite substantial functional deficits, but its capacity is limited and rapidly decreases with age. Nevertheless, neural and synaptic plasticity is required for functional integration of neural grafts and their activation could be one of the mechanisms of graft effect.

Second, the brain is a seat of memory, consciousness, and self-awareness, and it controls behavior, movements, as well as vegetative functions. All these processes are more or less based on specific cell-to-cell synaptic connections. If these specific circuits are altered by cell or fiber damage, full functional recovery would require precise reconstruction of the connections with all their complexity.

Third, grafting mature neurons is not possible because they do not survive the technical manipulation, nor can they migrate and integrate into the host's CNS circuits. Therefore, immature neural or stem cells must be grafted, which means that grafted cells must complete their development in the adult host's brain after transplantation (see Sect. 5). One advantage of this approach is that the immune response of the host CNS to grafted immature neural or stem cells is usually not very intense, so that in laboratory animals allografts survive for a long time even without any immunosuppression (Chintawar et al. 2009; Jones et al. 2010; Cendelin et al. 2012; Fuca et al. 2017).

Lastly, within the brain, the cerebellum represents a structure with its own specific features that may complicate neurotransplantation. Cerebellar function is based on complex circuits, microzones, and micromodules (Andersson and Oscarsson 1978; Ito 1984, 1990). Their complete reconstruction by grafted cells would be necessary for full specific functional restoration after the loss of any individual elements of these circuits and micromodules. In addition, the cerebellum has relatively low neurogenicity that may decrease survival of grafted cells and the capacity of stem cells to differentiate into specific local neuron phenotypes.

3 Mechanisms of Action of Cerebellar Transplants

Neurotransplantation therapy for cerebellar diseases can have several goals: specific cell substitution, rescue of degenerating cells, and support of residual cerebellar function. The goals could be hypothetically achieved via many diverse mechanisms, and

one mechanism can contribute to more than one goal. For instance, secretion of trophic factors and other bioactive molecules could help to rescue degenerating cells as well as support cerebellar function. Therefore, this mechanism is highlighted selectively as a relatively universal one having also impact on the fate of the graft itself.

3.1 Cell Substitution

Substitution of lost cells is in line with the classic view of transplantation therapy. The aim is to replace lost neurons with new ones, ideally in the same amount, having the same character and function. If this could be achieved, reduced cerebellar function would be fully restored. However, the outcome depends, among other factors, on the complexity of structures and functions to be restored. If replacement is only at the level of a neurotransmitter, there is a good chance of success. This is the case of Parkinson's disease (symptoms of which can be suppressed by pharmacological elevation of dopamine) but not the cerebellum, because the cerebellum contains densely packed populations of several neuronal types, each with different neurotransmitters. Moreover, grafted cells would need to survive long-term and differentiate into cerebellar-specific neurons as well as integrate into local circuits and reconstruct distal connections. In fact, cells grafted into the cerebellum would need to go through a process similar to embryonic development, but under different conditions and in a different niche, that is, the mature brain. In contrast to neuronal replacement, substituting injured glia, which do not subsequently have to reconstruct precise neuronal circuits, appears to be more successful: remyelination can be induced by injection of oligodendrocyte progenitors into the cerebellar white matter, as shown in a rat model of radiation-induced demyelination (Piao et al. 2015).

Thus, a key question for cerebellar transplantation, is what type of damage is to be treated (see Sect. 7) and whether one (and which) or several types of cerebellar neurons need to be substituted.

3.2 Cell Rescue

Rescuing host cells from degeneration or delaying degenerative process by grafted cells is a therapeutic mechanism that can be effective in early stages of slowly progressive pathologies, when a substantial portion of the host's own cerebellar cells still survives. Rescue of degenerating cells has been described, particularly by mesenchymal stem cell (MSC) grafts.

MSCs release a variety of paracrine factors, which are now considered to explain the primary therapeutic effect of transplanted MSCs on neuronal disorders (Jones et al. 2010; Nooshabadi et al. 2018). Accumulating evidence indicates that extracellular vesicles (EVs), released from MSCs, contain numerous substances (see Sect. 3.4) that exert neuroprotective effects in various murine models of neurological

disorders (Nakano and Fujimiya 2021) and that these molecules are transferred to damaged neurons and ameliorate their pathology.

Another mechanism to rescue degenerating neurons is cell fusion. Grafted MSCs have been shown to fuse with diseased Purkinje cells (Bae et al. 2007; Kemp et al. 2011; Diaz et al. 2012; Huda et al. 2016). The Purkinje cell ensures the specific neuronal cell phenotype, while the mesenchymal stem cell provides a healthy nucleus that is reprogrammed to produce Purkinje cell-specific proteins, and contributes to cell survival and normalization of its function (Kemp et al. 2018). However, there are doubts about long-term stability of these heterokaryons under physiological conditions (Nern et al. 2009) and the findings are contradictory (Weimann et al. 2003; Nern et al. 2009). However, the presence of pathological conditions in the host cerebellum is thought to increase the frequency of cell fusion (Diaz et al. 2012; Huda et al. 2016; Kemp et al. 2018). All in all, animal model studies have suggested that cell fusion could effectively provide the host's cerebellum with surviving and functioning Purkinje cells. Nevertheless, evidence for long-term persistence of such heterokaryon is needed to promise stable effects in human patients.

In contrast to MSCs, grafted neural stem cells (NSCs) also support degenerating cells, but without fusing with them. Instead NSCs use gap-junction connections to provide metabolic support for deteriorating cells (Jaderstad et al. 2010).

3.3 Support of Residual Cerebellar Function

Another aim of neurotransplantation is to support residual cerebellar function. The graft can potentiate cerebellar reserve, defined as the capacities of compensation and restoration for pathological tissue damage (Cendelin et al. 2018a, 2019; Mitoma et al. 2020, 2021). Cerebellar ataxias (CAs) induced by short-lived pathologies, such as stroke or trauma, can show partial and sometimes complete recovery with time (Mitoma et al. 2020). In these cases of sudden structural damage in a limited area of the cerebellum, the ensuing functional cerebellar deficit can be restored through compensation by other areas not affected by the lesion, and thus this is termed structural cerebellar reserve (Mitoma et al. 2020). On the other hand, in slowly progressive and often controllable pathologies, such as immune- or metabolic/toxic-mediated CA, elimination of the insult subsequently results in partial or complete recovery (Mitoma et al. 2020). In these diffuse disorders, the affected tissue itself may maintain diminishing cerebellar functions if sufficient neurons survive and sufficient cerebellar microcomplexes remain functioning, which is termed functional cerebellar reserve (Mitoma et al. 2020). In other words, the manipulation of cerebellar reserve should prevent the functional progression of degenerative diseases.

The cerebellar reserve correlates with specific cerebellar functions involved in acquiring and updating the internal forward model, the cerebellar circuitry being the neural substrate of state predictions (Mitoma et al. 2021). The cerebellum integrates multimodal cerebral and peripheral inputs through multiple plastic modifications at divergent synapses to acquire the internal model, which is used to adapt to the

environmental changes inherent to daily life (Mitoma et al. 2021). Since the aim of any therapy should be to preserve or enhance cerebellar function, using neurotransplantation to increase this reserve could represent a central therapeutic strategy in the near future.

If neurotransplantation is introduced during a stage when cerebellar reserve is still relatively preserved (not advanced degeneration), greater therapeutic effects can be expected (see Sect. 7). At an early disease stage, multimodal cerebellar inputs and multiple forms of synaptic plasticity are still present. It is, therefore, anticipated that neurotransplantation reorganizes the damaged cerebellar circuits in order to render functions effective again. In degenerative CA, neurotransplantation in asymptomatic or prodromal stages might be a promising therapeutic strategy. Importantly, such asymptomatic and prodromal stages have been described both in animal models (Chen et al. 2020) and human degenerative CA (de Oliveira et al. 2021; Velázquez-Pérez et al. 2021), in which gait instability and ocular deficits occur. It is now obvious that there is a need for early therapeutic intervention and development of morphological and functional biomarkers for cerebellar reserve.

3.4 Provision of Trophic Factors and Other Molecules Produced by Grafted Cells

An important mechanism mediating cell rescue and support of residual cerebellar function is the molecules released by grafted cells such as trophic factors and compounds contained in EVs. These substances together with molecules produced by the host's cells determine the local tissue niche that influences not only the state of residual intrinsic cells, and their function, but also the graft and graft-derived cells (for details see Sect. 5).

EVs released from MSCs contain DNA, mRNA, microRNA (miRNA), proteins, and mitochondria (Spees et al. 2006; Lai et al. 2014; for review see Lai and Breakefield 2012). Since depletion of some miRNAs, which suppress the expression of numerous target genes, is thought to trigger neurodegeneration (Schaefer et al. 2007; Yuva-Aydemir et al. 2011; Roshan et al. 2012), complementing such miRNAs by those from MSCs/EVs may suppress cell death. Meanwhile, brain-derived neurotrophic factor (BDNF) has been shown to play a key role in survival and dendritic differentiation of Purkinje cells (Hirai and Launey 2000; Hisatsune et al. 2006) as well as cerebellar plasticity (Carter et al. 2002; Sadakata et al. 2007), and BDNF levels decrease in SCA1-transgenic mice (Mellesmoen et al. 2018). Thus, BDNF released from MSCs may be the therapeutic component of MSC grafting (Jones et al. 2010; Sivandzade and Cucullo 2021). Also transfer of functional mitochondria from MSCs via EVs or other routes can maintain survival of degenerating host's cells (for review see Torralba et al. 2016; Paliwal et al. 2018; Liu et al. 2020).

In addition, migration of graft-derived cells is important for appropriate functional integration. Purkinje cell migration is controlled not only by molecules such as reelin and tenascin (Goffinet 1983) but also by glial-derived neurotrophic factor (GDNF) (Sergaki and Ibanez 2017). Trophic factors promote axon growth and insulin-like growth factor 1 (IGF-1) and BDNF have been shown to induce reinnervation of Purkinje cells and cerebellar nuclear neurons after olivocerebellar pathway lesion (Dixon and Sherrard 2006). Thus, graft cell-derived trophic factors are likely to facilitate host neuron survival and functional reserve (but see Sect. 5.3).

Glial cells are also an important source of molecules that modulate the local tissue niche and its neurotrophic features, such as GDNF or sonic hedgehog. It has been shown that grafting astrocytes induced neurogenesis in the non-neurogenic neocortex in mice (Jiao and Chen 2008).

Taken together, substances produced by the graft can modify the pathological processes, rescue degenerating cells and increase survival of the graft itself, promote neural plasticity, and thereby stimulate both residual tissue function and functional integration of the graft. On the other hand, delivery of extrinsic factors or modulation of their local expression may by itself induce similar effects.

4 Graft Sources and Types

Many types of cells have been tested as grafts in experiments in laboratory animals. All have some advantages, some even being effective in animal models of neurological diseases, but all of them have their disadvantages or serious limitations (including technical and ethical) complicating their routine effective and safe use in clinical practice.

4.1 Fetal Cerebellar Tissue

Fetal cerebellar tissue has been grafted into the cerebellum of diverse mouse models of cerebellar degeneration in many studies. It survives for a long time in the host's cerebellum, and is a good source of Purkinje cells (Sotelo and Alvarado-Mallart 1987; Triarhou et al. 1987; Tomey and Heckroth 1993; Cendelin et al. 2009; Purkartova et al. 2014; Babuska et al. 2015; Fuca et al. 2017; Cendelin et al. 2018b; Purkartova et al. 2019). Theoretically, it could be one of the approaches to substitute lost cells. Nevertheless, therapeutic benefit is still debatable (see Sect. 6.1). The main problem, however, is the source of the graft, which for human clinical use would be aborted human fetuses. Besides serious ethical problems, poor standardization of graft quality or risk of infection are complicating issues.

4.2 Mesenchymal Stem Cells

MSCs, multipotent progenitor cells, can be isolated from various tissues such as bone marrow, adipose tissue, umbilical cord, and placenta without any substantial risk or damage to the donor. The MSCs can be cultured and propagated in vitro. Thus, there are mostly no ethical limitations. Autotransplantation of MSCs is also possible offering full antigen compatibility. However, when grated into the cerebellum, only a limited number of MSCs are able to differentiate into the specific neurons or glia and replace the damaged cells (Bae et al. 2007; Jones et al. 2010; Chang et al. 2011; Matsuura et al. 2014). Therefore, MSCs do not currently appear to be a good tool for specific cell substitution. On the other hand, these cells possess anti-apoptotic, neuroprotective, immunomodulatory, and anti-inflammatory properties (Bae et al. 2010; Jones et al. 2010; Nooshabadi et al. 2018), as well as the ability to fuse with degenerating cells (Bae et al. 2007; Huda et al. 2016). They therefore have potential to alleviate a wide range of diseases, including inflammatory, immune-mediated, and degenerative diseases (Saeedi et al. 2019). See also Sects. 3.2 and 3.3.

Although many beneficial influences of MSCs have been reported, we should keep in mind the potential nature of MSCs to transform into cancer stem cells (Li et al. 2021) or to promote tumor progression by secretion of various tumor-promoting factors, such as growth factors, anti-apoptotic and immunomodulatory molecules (Djouad et al. 2003; Karnoub et al. 2007; Ramasamy et al. 2007; Beckermann et al. 2008; Tsai et al. 2011; for review see Ahn 2020), some of which are likely to be necessary for the therapeutic effects of the graft.

4.3 Embryonic, Carcinoma, Adult, and Induced Pluripotent Stem Cells

Besides MSCs, many other types of stem cells are available for investigation. The general idea is that they would be multiplied in vitro, treated with biologically active substances to induce differentiation to certain neural or neuronal phenotype and grafted to the patient in preclinical studies represented by an animal model. Recently, thanks to biotechnology advances, there are protocols allowing the generation of cerebellar-specific neurons in vitro, with increasing yield (Muguruma et al. 2015; Watson et al. 2018; Nayler et al. 2021). Such cells could become material for specific neurotransplantation therapy in the future, substituting lost cerebellar neurons. However, the cells must be grafted in an immature state, as mentioned above, and the question still remains of what is the optimum timepoint when these cells are sufficiently mature not to redifferentiate into other cell types, while still retaining features of stem or immature precursor cells that are necessary for successful survival, migration, fiber sprouting after engraftment.

Carcinoma stem cells are easy to culture in vitro. Neural progenitors derived in vitro from these cells are effective in experimental therapy in animal models of amyotrophic lateral sclerosis (Garbuzova-Davis et al. 2002), Parkinson's disease (Baker et al. 2000), spinal cord injury (Saporta et al. 2002), and stroke (Hara et al. 2007). There is little information about transplantation of carcinoma stem cell-derived grafts into the cerebellum. In the healthy mouse cerebellum or the cerebellum of Lurcher mice, carcinoma stem cell-derived neuroprogenitors did not integrate into the host's cerebellum and did not adopt Purkinje cell phenotype (Houdek et al. 2012), so that successful generation of cerebellar neurons from carcinoma stem cells has not yet been reported. Although some authors suggest that after induction of neurodifferentiation, carcinoma stem cells become postmitotic (Pleasure et al. 1992; Garbuzova-Davis et al. 2002), they are considered dangerous for use in patients because of their tumorigenic potential.

Neural stem cells can be isolated from donor brains, particularly from neurogenic areas, such as the subventricular zone or immature brains. Thus, gain of these cells is connected with death of the donor (abortion material in humans) like in the case of fetal neural tissue and thereby ethically unacceptable.

While several studies reported that NSCs do not tend to adopt cerebellar neuronal phenotypes (Chintawar et al. 2009; Rolando et al. 2010; Tailor et al. 2013; Mendonca et al. 2015), other studies showed that differentiation into granule cells and rarely also Purkinje cells is possible (Rosario et al. 1997; Lee et al. 2005). NSCs stem cells have been shown to form gap junctions with degenerating host's cells and provide metabolic support that could delay degeneration process (Jaderstad et al. 2010) (see Sect. 3.2).

Embryonic stem cells can be maintained and propagated effectively in vitro and theoretically have the capacity to differentiate into various cell types. Muguruma et al. (2015) developed a protocol to generate cerebellar organoids from embryonic stem cells. Nevertheless, routine use of embryonic stem cells harvested from human embryos is ethically problematic.

Induced pluripotent stem cells (iPSCs) are a relatively new material for potential cell therapy (Takahashi and Yamanaka 2006). They can be derived from various tissues without any significant damage to the donor and so their resources are ethically acceptable and can offer autologous grafts. There are still many questions that remain to be answered regarding efficiency of their generation, differentiation into particular cell types, and efficiency and safety of their potential use for neurotransplantation therapy. Nevertheless, cerebellar Purkinje cells or progenitors have already been successfully generated from iPSCs (Wang et al. 2015; Watson et al. 2015, 2018) and it has been shown that the iPSC-derived Purkinje cell progenitors can survive when grafted into the mouse cerebellum (Wang et al. 2015). iPSC-derived cerebellar organoids have been shown to recapitulate cerebellar development and thus they have potential to become a tool for reconstruction of damaged cerebellar tissue (Nayler et al. 2021).

5 Graft Survival, Differentiation, Migration, and Axon Growth in the Host Tissue

For appropriate functional effect of the graft, its survival and development in the host's cerebellum are key. To achieve this goal, several factors are essential: survival of a sufficient number of grafted cells, their differentiation into cerebellar-specific phenotypes that are to be substituted, and their appropriate synaptic integration into the host's cerebellar circuits. Nevertheless, long-term graft survival and differentiation may not be necessary if the purpose of the graft is only to provide transient support of neural plasticity or intrinsic stem cell proliferation (Kumar et al. 2014).

5.1 Factors Determining Graft Survival

An important factor determining survival of cells immediately after engrafting is any injury (mechanical, chemical, hypoxia, etc.) occurring during graft preparation (cell harvesting, cell suspension preparation) and the grafting procedure itself. To this can be added injury of the host tissue caused by graft injection, which also produces local hypoxia, ion imbalance, and reactive oxidative species. In turn, local tissue damage could induce local inflammation, modifying tissue milieu, thus curtailing the effectiveness of grafted material. Further, allogeneic or xenogeneic transplantation faces the challenge of rejection due to host-to-graft immune response. All these factors are always present to greater or lesser extent and can only be reduced by technical and procedural refinement. Here, we focus on graft cell survival in the horizon of days, months, and years. Such long-term survival depends on properties and viability of grafted cells (their type, differentiation stage) and on local niche of the host's tissue, in this case an unhealthy pathological cerebellum.

Survival of cerebellar precursors after transplantation is mainly determined by appropriate matching of the transplanted cells and host species. Isografting cerebellar neural precursors into the developing (Carletti et al. 2002) or adult cerebellum (Sotelo and Alvarado-Mallart 1986) of mice and rats is well tolerated and leads to excellent survival of the grafted cells. However, in vivo modeling of neurogenetic and neurodegenerative diseases is often dependent on human stem cell-derived xenografts (Kemp et al. 2011; Tailor et al. 2013; Piao et al. 2015; Wang et al. 2015; Huda et al. 2016; Li et al. 2018; Tsai et al. 2019). The immune-reaction induced by xenotransplantation into the cerebellum of immunocompetent hosts severely limits transplant survival (Nato et al. 2021) and strategies to control rejection must be implemented if long-term survival of the transplant is the goal. However, both immunosuppressive therapy, with CyclosporineA6 or tacrolimus, and grafting into a severely immunodepressed host are limited when graft survival must exceed few months (Brundin et al. 1985; Strömberg et al. 1988; Tamaki et al. 2002; Itakura et al. 2015).

An alternative approach to successful xenotransplantation is inducing immunotolerance of the xenotransplant cell-of-interest *before* maturation of the host immune system (Kelly et al. 2009). Unfortunately, immunotolerance induction results in wide variations in survival of the neural graft according to the species and the strain of the host (Mattis et al. 2014), the species of the donor (Janowski et al. 2012; Jablonska et al. 2013), and even the region in the brain receiving the xenograft (Fainstein and Ben-Hur 2018). Xenograft of cerebellar neural precursors into the developing cerebellum in utero results in unlimited survival and differentiation of the transplanted cells when the duration of development of the donor and host species is comparable (Magrassi et al. 2013). However, when there is considerable difference in developmental duration, as it is the case when human neural precursors are transplanted into the developing rodent cerebellum, the transplant is invariably rejected after a relatively short period (approximately 30 days) of survival and differentiation (Nato et al. 2021). In this case, the slower pace of differentiation of human neural precursors, compared to that of rodents, limits immune-tolerance to those human antigens expressed by the transplanted cells before the host immune system matures, while new antigens expressed after maturation of the host immune system are not recognized and rejected (Nato et al. 2021). In this experimental paradigm, survival of the cerebellar xenograft was tripled by adding a mature rat cerebellar extract to the human cellular suspension grafted into a developing mouse cerebellum (Nato et al. 2021). These results suggest that antigens present in the adult rat cerebellum are sufficiently homologous to those of more differentiated human neural cells to enhance immuno-tolerance in the host mice. These findings may inform current efforts in the development of hypoimmunogenic pluripotent stem cell lines for universal transplantation purposes based on inactivation of histocompatibility complex genes (Aron Badin et al. 2019; Deuse et al. 2019; Han et al. 2019).

Host immune reaction against the graft is an important but not exclusive determinant of graft survival. There is a growing awareness that xenografting human cells or tissues into severely immune-deficient rodents (e.g., SCID mice and rats) may not be optimal for modelling the presumptive behavior of these in clinical transplants. This is because, in only a very few days, the host mouse virome replaces the virome typical of the donor grafted cells before transplantation. Replacement or mixing of the donor and host virome may have profound effects on the transplant's expression of many genes related to differentiation, immunity, and drug metabolism, which ultimately modify graft survival (Yuan et al. 2021).

In addition to the key host response of immune-rejection, other factors specific to the cerebellum influence the survival of grafted cells. There are strong indications that the environment of the host cerebellum, especially in disease states, plays an important role in the morphological organization of the graft and its connection with the host cerebellum (Purkartova et al. 2019). The signals from the diseased cerebellum may be negative (Cendelin et al. 2018b; Purkartova et al. 2019), positive (Carletti and Rossi 2005) or have no effect when compared with the healthy cerebellum (Fuca et al. 2017; Higuera et al. 2017). For example, the cerebellum of Purkinje cell degeneration mice provides strong signals to promote survival of grafted Purkinje cells (Carletti and Rossi 2005).

Furthermore, pathological processes which damage the cerebellum can equally damage the graft. This strongly depends on the etiology including its character and persistence. Typical examples include persisting ischemia, toxins including intrinsic toxic substances and local metabolites, as well as systemic metabolic disturbances, and chronic inflammation including autoimmunity. Yet, neurodegenerative processes can also affect both the host's tissue and the graft. Alpha-synuclein-positive Lewy bodies developed in grafted neurons in Parkinson's disease patients treated with neurotransplantation (Kordower et al. 2008; Li et al. 2008). It has been shown that alpha-synuclein can be transmitted via endocytosis to neighboring neurons, which explains the spread of the neuropathology from primarily affected host's neurons to grafted cells (Desplats et al. 2009). In cerebellar degenerations, such information is lacking since there are few clinical trials documented in details.

5.2 Differentiation of Grafted Cells

The ideal source of replacement cells for clinical grafts in humans would be aborted human fetuses, because they contain properly specified precursors with the full potential to differentiate into the relevant neuron types and support lost functions. However, several issues, including ethical problems (see also Sect. 4.1) and the need for appropriate scaling of tissue quantity for translation to regenerative therapies, make the use of human fetuses difficult; thus, prompting the development of in vitro protocols to obtain cerebellar neurons from human stem cells is important. Building on knowledge of embryonic cerebellar development and studies on rodent pluripotent cells, the most efficient protocols aimed primarily at mimicking the self-inductive properties of the isthmic organizer to activate endogenous signaling cascades and obtain cerebellar progenitors (Muguruma et al. 2010; Wang et al. 2015; Watson et al. 2018). To promote the generation of human Purkinje neurons, human cerebellar progenitors have been exposed to instructive paracrine and/or juxtacrine factors through coculture with either rodent embryonic cerebellar cells or human cerebellar slices (Muguruma et al. 2010; Wang et al. 2015; Ishida et al. 2016; Watson et al. 2018). Overall, these protocols yield between 10% and 90% maturing Purkinje neurons, depending on the implementation of procedures for enrichment of Purkinje neuron progenitors (Tochitsky et al. 2018; Watson et al. 2018). The high efficacy of in vitro differentiation protocols is important, not only to ensure generation of sufficient specific cerebellar neurons, but also to reduce grafted cells that could subsequently adopt non-cerebellar phenotypes and occupy cerebellar structures.

Also, three-dimensional self-organizing organoids derived from pluripotent stem cells offer a model to promote Purkinje cell differentiation without the need of mouse co-cultures (Muguruma et al. 2015; Nayler et al. 2021), and to obtain other cerebellar cell types including rhombic lip derivatives and cerebellar interneurons (Muguruma et al. 2015; Nayler et al. 2021). Moreover, Matrigel embedding, to mimic basement membrane positional signaling, altered growth dynamics of the cerebellar organoids and influenced organoid composition through biasing lineage commitment towards rhombic lip formation (Nayler et al. 2021).

All these approaches are valuable for disease modelling in order to understand mechanisms underlying cerebellar ataxia and drug screening. These methodologies will be particularly powerful when combined with other cutting-edge technologies including genome engineering, single-cell sequencing, and high-throughput microscopy. Moreover, additional advances in generating neurons of specific subtypes will likely be stimulated by single cell/nuclei transcriptional and epigenetic profiling of the developing human and rodent cerebellum (Aldinger et al. 2021; Sarropoulos et al. 2021) in combination with forward reprogramming of human neural progenitors or somatic cells. The importance of this approach is underlined by the relatively low neurogenic potential of the host cerebellum, which does not promote differentiation of grafted stem cells into cerebellar neurons (Chen et al. 2009; Chintawar et al. 2009; Rolando et al. 2010; Tailor et al. 2013; Mendonca et al. 2015). Moreover, stem cells that do become cerebellar-specific neurons generate granule cells rather than Purkinje cells (Rosario et al. 1997; Lee et al. 2005).

However, advancements are required for the development of realistic human therapies based on neurotransplantation. In particular, significant research remains to be undertaken in three areas. Firstly, it is essential to ensure reproducibility of protocols generating large quantities of properly specified cerebellar neurons. Secondly, confirmation of non-contamination by tumorigenic or unwanted neural types, as well as no risk for genomic instability is needed. Lastly and importantly, methods of graft preparation, in vitro culture and treatment to induce specific cell differentiation substantially influence their fate after engraftment. These methods are under intensive investigation and currently, progress is rapid. However, in vivo preclinical studies are currently lacking and should be performed to define the best time point in vitro for grafting of human committed progenitors and fully phenotype transplanted human cerebellar neurons, through extensive and long term transcriptional, neurochemical, anatomical and functional evaluation. In addition, proliferation activity of the cells both in vitro and in vivo after engraftment is of importance. Intensive proliferation provides more cells but uncontrolled proliferation could convert the graft into an expansive intracranial process or even a real tumor. Adequate in vitro treatment is crucial to balance proliferation activity and keep it under control for safety of the therapy.

5.3 Migration and Synaptic Integration of Grafted Cells

In addition to the survival and differentiation of graft cells, they need to integrate into the host cerebellar circuitry before they can have beneficial functional effects. Importantly, both poor and over-abundant migration of graft cells can be a problem, and this migration is affected by the host cerebellar tissue. Grafted cells in incorrect positions could theoretically disturb local circuitries and functions. Particularly, MSCs showed ability to migrate through the organism (Crain et al. 2005; Chang et al. 2011). Nevertheless, mostly inappropriate migration is discussed in terms of its negative impact on synaptic integration of grafted cells and failure to reconstruct cerebellar circuitries.

For example, the Lurcher mutant cerebellum reduces cell migration and fiber sprouting from the graft in comparison with wild-type tissue (Cendelin et al. 2018b; Purkartova et al. 2019). Moreover, cellular integration also depends on the cell type. Grafted granule cells generated in vitro from embryonic stem cells do incorporate into the host's granular layer and form synaptic contacts with mossy fibers (Salero and Hatten 2007). However, the integration of Purkinje cells is less effective. These grafted cells form normal cerebellar cytoarchitecture only with other graft cells, and not with surviving host cells (Rosenfeld et al. 1993). Moreover, graft Purkinje cells cannot extend their axons from their molecular layer location toward the deep cerebellar nuclei through the barrier of the granular layer (Keep et al. 1992; Carletti et al. 2008), in contrast to grafts in other CNS regions that do extend axons to their targets (Kikuchi et al. 2017). This lack of connectivity is a severe limitation to the functional benefit of cerebellar Purkinje cell transplantation. Consequently, graft injection under the cerebellar cortex has been recommended to achieve graft Purkinje cell-to-deep nuclear neuron connectivity, but this would complicate subsequent graft synaptogenesis with parallel fibers and molecular layer interneurons (Keep et al. 1992). However, adjunct treatment with the trophic factor GDNF, which facilitates Purkinje cell migration (Sergaki and Ibanez 2017), may offer future potential to overcome this current difficulty.

Graft Purkinje cell integration also requires dendritic development to receive parallel and climbing fiber synapses, but grafted Purkinje cells are often mis-oriented, so their dendrites are not immediately adjacent to parallel fiber afferents. Nevertheless, Purkinje cell dendritic growth is principally an intrinsic growth program (Kapfhammer 2004; Sotelo and Dusart 2009) that is promoted by trophic factors such as IGF-1 (Torres-Aleman et al. 1994; Nieto-Bona et al. 1997) and BDNF (Carter et al. 2002; Sadakata et al. 2007). These two factors are expressed by granule cells throughout life (Borghesani et al. 2002; Tsutsui et al. 2011), thus allowing structural and functional synaptic plasticity. For example, in the presence of unoccupied Purkinje cell spines, such as those that develop on grafted Purkinje cells, mature parallel fibers are able to sprout, developing new terminal branches and synapses to fill the available space (Chen and Hillman 1982). Indeed, the synthesis of BDNF by mature granule cells will facilitate spine production on the maturing graft Purkinje cell dendritic tree (Shimada et al. 1998), which expresses TrkB receptors (Carter et al. 2002). In contrast, climbing fibers of the mature cerebellar cortex are much less plastic than parallel fibers. While they are capable of sprouting within the molecular layer to reinnervate adjacent denervated Purkinje cells, including grafted Purkinje cells (Tempia et al. 1996), the overall effect is local and is limited to a maximum of 100 μm (Rossi et al. 1991; Dhar et al. 2016), which will reduce graft Purkinje cell innervation. However, IGF-1 and BDNF greatly increase climbing fiber growth and Purkinje cell reinnervation (Sherrard and Bower 2003; Dixon and Sherrard 2006), suggesting that adjunct growth factor treatment may facilitate graft integration into the existing cerebellar cortical circuit (see Sect. 3.4).

Taken together, these studies indicate that graft cell integration into the host cerebellar circuitry is possible, especially for receiving host afferent parallel and climbing fiber input. However, the major challenge still to be met is the induction of functional graft Purkinje cell output to cerebellar deep nuclei and the rest of the brain.

6 Examples of Mouse Model Studies

Cerebellar transplantation has been studied for many years in various types of mouse models and using many types of grafts and modes of administration. The experiments have generated a lot of information which is both positive and negative.

6.1 Grafting Fetal (Embryonic) Cerebellar Tissue

Fetal cerebellar tissue transplantation is the classical approach that has been investigated in many mouse models (Fig. 1). Early studies in 80s and 90s of the twentieth century showed good survival of this type of graft and in some cases, mainly Purkinje cell degeneration and Lurcher mice, also colonization of the host's cerebellum by graft-derived Purkinje cells (Keep et al. 1992; Dumesnil-Bousez and Sotelo 1993; Tomey and Heckroth 1993). Also in Weaver mice, grafted embryonic cerebellar cells created a structure with typical cerebellar trilaminar organization and graft-derived granule cells have been reported to develop synaptic contacts (Takayama et al. 1987, 1988; Kohsaka et al. 1988).

However, there was no consensus on graft effect on motor performance in ataxic mice treated with this kind of graft. Some studies in Purkinje cell degeneration and SCA1 mice showed improvement (Triarhou et al. 1995, 1996; Kaemmerer and Low 1999) but some did not or found only mild and unconvincing effect, e.g., in

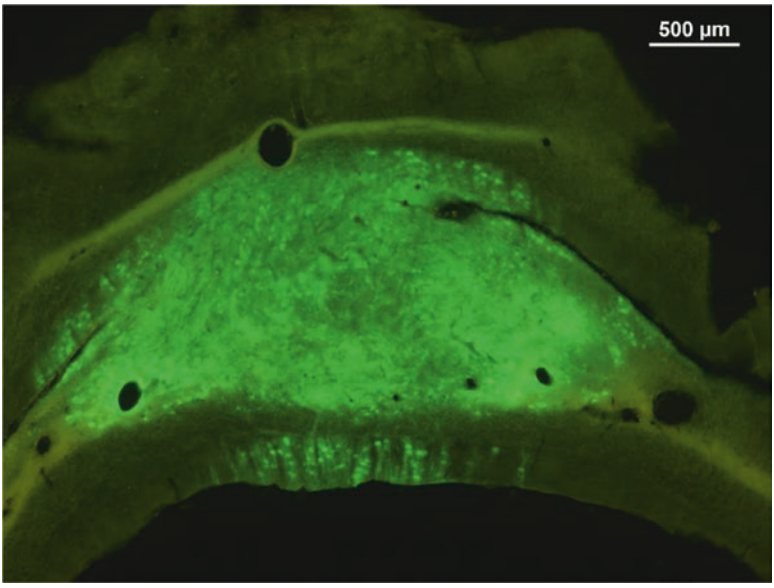

Fig. 1 A green fluorescent fetal cerebellar graft in the mouse cerebellum

tambaleante, Lurcher and also Purkinje cell degeneration mice (Babuska et al. 2015; Fuca et al. 2017; Cendelin et al. 2018b; Purkartova et al. 2019).

6.2 Neural Precursor or Neural Stem Cell Transplantation

More recently, stem cell-derived neural precursors or neural stem cell transplantation has become the focus of interest. Neural precursor transplantation rescues degenerating Purkinje cells by reducing excessive tissue plasminogen activator level and thus improved motor performance in nervous mutant mice (Li et al. 2006). In SCA1 mice, grafted neural progenitors increased survival of the host's Purkinje cells and improved motor performance (Chintawar et al. 2009). This type of graft has been shown to reduce neuropathology and motor impairments also in SCA3 and Niemann-Pick type C disease mouse models (Ahmad et al. 2007; Lee et al. 2010; Mendonca et al. 2015).

6.3 Cerebellar Parenchymal Injection of Stem Cells

Since the year 2000, intracerebellar transplant of stem cells, mostly MSCs, has been extensively studied. Cerebellar parenchymal administration of MSCs is effective in reducing Purkinje cell death, but through different mechanisms according to the underlying pathology. In SCA3-transgenic mice (Oliveira Miranda et al. 2018) and Niemann-Pick type C mice (Bae et al. 2005, 2007, 2010), transplant of mouse bone marrow-derived MSCs alleviated the pathological process to reduce Purkinje cell death and maintain motor control. MSC transplant into the newborn Lurcher cerebellum also delayed Purkinje cell degeneration and reduced cerebellar ataxia, while the MSCs were found close to the Purkinje cell layer, where they produced neurotrophic factors (Jones et al. 2010).

Similarly, xenografting human umbilical cord MSCs significantly mitigate pathological progression in SCA1-transgenic mice, in terms of Purkinje cell degeneration and motor behavior (Tsai et al. 2019).

6.4 Intravenous or Cerebroventricular Infusion of MSCs

Recently, several studies have shown that systemic administration of MSCs might be an effective solution. When MSCs expressing luciferase were intravenously injected to SCA3 model mice, their bioluminescence was detected in the brain 30 min after administration (Oliveira Miranda et al. 2018). Also, in mice sacrificed at this 30 min timepoint, cerebellar immunohistochemistry showed MSCs in the cerebellar parenchyma, confirming that MSCs leave the cerebral blood vessels to

reach the degenerating cerebellar tissues (Oliveira Miranda et al. 2018). However, the MSC bioluminescence had almost disappeared 48 h after intravenous administration.

Similarly, when MSCs were injected to the brain lateral ventricle of the brain, they stayed in the ventricle and the MSC graft volume rapidly decreased from 1 to 4 weeks (Oliveira Miranda et al. 2018). Consistent with this observation, clinical trials show that the beneficial effect of MSCs is only transient (Dongmei et al. 2011; Jin et al. 2013). Thus, a major problem of MSC transplantation for SCA is their short post-transplant life, in contrast to the ongoing production of toxic substances in the SCA cerebellum. To overcome this short survival time, Oliveira Miranda et al. (2018) and Li et al. (2018) tested repeated MSC intravenous administration finding that it increased cerebellar trophic factors and modulated the level of 70 kD heat shock protein (Li et al. 2018), reducing the neuropathology and conferring improved motor function (Oliveira Miranda et al. 2018).

Similarly in Purkinje cell degeneration mice, although a single intravenous MSC injection improved motor performance, Purkinje cell rescue required repeated daily intravenous injections, consistent with this aggressive cerebellar degeneration (Díaz et al. 2019). In this case, Purkinje cells survived through fusion with the grafted stem cells (Díaz et al. 2019).

6.5 Administration of Stem Cell Products

A major problem of intravenous MSC transplantation is that a large fraction of MSCs will be trapped in the lung, resulting in only a limited portion of MSCs reaching the brain (Oliveira Miranda et al. 2018) and thus compromising the MSC therapeutic potential. Alternatively, MSC-derived products, such as EVs or exosomes released from MSCs (You et al. 2020), conditioned medium of the MSC culture (Suto et al. 2016) or pure trophic factors (Sheeler et al. 2021) could be infused. For example, intravenous infusion of exosomes, which were released from induced pluripotent stem cell-derived MSCs, blocked Purkinje cell apoptosis in SCA3-transgenic mice, resulting in significant improvement of motor function (You et al. 2020). Although such MSC-derived factors are diluted in the peripheral circulation, reducing their efficacy, these infusions can be repeated. Watase et al. have tested the therapeutic effect of MSC-conditioned medium on SCA1-knock-in (SCA1-KI) mice, which have a relatively weak phenotype (Watase et al. 2002). Presymptomatic SCA1-KI mice aged 4 weeks received a single intrathecal injection of the MSC-conditioned medium, followed x10 intravenous conditioned medium infusions every 2 weeks up to 24 weeks of age (Suto et al. 2016). This treatment significantly reduced the SCA1 pathology.

Suzuki et al. then tested the therapeutic effect of the same MSC-conditioned medium on a polyglutamine disease model with a severe phenotype, dentatorubral-pallidoluysian atrophy (DRPLA) transgenic mouse, expressing mutant atrophin-1 with 113 polyglutamine repeats (Suzuki et al. 2012). Similar to SCA1-KI mice,

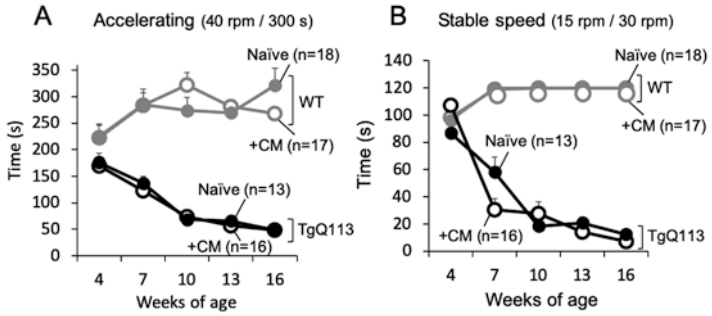

Fig. 2 No beneficial influence of MSC-conditioned medium on progressive motor defect of DRPLA mice. Wild-type and DRPLA mice received intrathecal MSC-conditioned medium at 4 weeks of age, followed by intravenous infusion of the same medium every 2 weeks. The motor ability of the mice was examined by rotarod every 3 weeks (4, 7, 10, 13, and 16 weeks of age). (**a**, **b**) Rotarod results obtained from wild-type (WT) and DRPLA (TgQ113) mice treated with (+CM) or without (naïve) the conditioned medium. The mice were tested using accelerating 40 rpm in 300 s; (**a**) and stable 30 rpm for WT mice and 15 rpm for DRPLA mice; (**b**) speeds

4-week-old DRPLA mice received a single intrathecal injection followed by intravenous infusions (every 2 weeks) of the conditioned medium. In contrast to the case of SCA1-KI mice, however, the MSC-conditioned medium failed to suppress the progressive behavioral deterioration (Fig. 2). Thus, MSC-conditioned medium seems to have clear, but currently, only modest therapeutic effect on polyglutamine disease pathology. Since many beneficial factors released from MSCs, which could mediate therapeutic influence on diverse types of neurological disorders, have been proposed (Tsai et al. 2019; Nakano and Fujimiya 2021; Sivandzade and Cucullo 2021), identification of MSC-derived factors and/or miRNAs, which play key roles in mitigation of SCA pathology, and their enriched and repetitive application may achieve a more robust therapeutic outcome.

7 Comments on Clinical Applications of Neurotransplantation for Cerebellar Ataxias

Neurotransplantation has been shown to be relatively effective in therapy of the Parkinson's disease (Lindvall 2015; Parmar 2018). Clinical studies have been done also in Huntington's disease patients (Kumar et al. 2020) and multiple system atrophy (Lee et al. 2008; Dongmei et al. 2011; Xi et al. 2013), the latter including cerebellar pathology and symptomatology. Nevertheless, there are only few studies in patients with cerebellar degeneration (Wu et al. 1991; Tian et al. 2009; Dongmei et al. 2011; Jin et al. 2013). Specifically for SCA3, a clinical trial on six patients showed good tolerance and slightly optimistic results of intravenous MSCs infusion with 12 months follow-up (Tsai et al. 2017). Although these studies reported positive results, they are not sufficient to provide a guarantee of acceptable risk/benefit

ratio of neurotransplantation as a routine therapy for human cerebellar patients. Therefore, further controlled, randomized, double-blinded studies recruiting sufficient number of patients with precisely defined type and stage of cerebellar pathology and with long follow-up are needed for individual SCAs and other cerebellar diseases. Also, there are many questions to be answered by pre-clinical animal model-based studies (see Part 6).

Neurotransplantation is, indeed, an invasive procedure and potential serious adverse events have to be taken into account. A potential problem common to all immature cell grafts is, for instance, their potential to re-derive into tumor cells (Amariglio et al. 2009). In most CAs, neurotransplantation is not and cannot be the sole envisioned therapy. Functional access to the cerebellar reserve could potentially be achieved with other approaches. Thus, it is plausible to consider that a multimodal approach is more realistic. For wide and routine use of neurotransplantation therapy, it should be shown not only that it is efficient but also that it has better negative side effect/benefit ratio than other approaches. This ratio could, however, be different for different cerebellar diseases and different stages of the respective disease and therefore the optimum therapy may not be universal.

A first feasible option is non-invasive cerebellar stimulation which promotes synaptic plasticity and, therefore, has great potential for the reconstruction of lost cerebellar motor as well as cognitive functions (Ferrucci et al. 2019; Manto et al. 2021) as already shown by clinical studies (Benussi et al. 2015, 2017, 2021).

Targeting pathogenetic processes is another promising therapeutic approach in degenerative diseases of the cerebellum. Down- or up-regulation of the expression of the target pathogenic gene or molecular-targeted therapy has been reported to halt disease progression and rescue cells from cell death in animal experiments (Chen et al. 2008; Boy et al. 2009; Liu et al. 2009; Furrer et al. 2013; Chort et al. 2013; Nobrega et al. 2013; Rodriguez-Lebron et al. 2013; Wang et al. 2013; Ramachandran et al. 2014). However, both the efficacy and safety of such therapies for individual types of cerebellar diseases have not yet been verified.

It should be underlined that cerebellar disorders comprise a myriad of diseases. When considering neurotransplantation for therapy, many factors must be analyzed (Cendelin et al. 2019). Unless effective less-invasive therapy is available (e.g., for immune-mediated CAs), important aspects are the character and stage of the disease. For each pathology and disease progression stage, different types of grafts would be needed to provide appropriate cellular mechanisms (see Sect. 3) that allow the graft to be functionally effective (Cendelin et al. 2018a, 2019). For example, in the early stages of slowly progressive cerebellar degeneration, when substantial numbers of cerebellar neurons still survive, a cell-rescue approach might be most appropriate if the pathological process is controllable. On the other hand, in sudden or rapidly developing pathologies, such as trauma or vascular disorders, cerebellar neurons die rapidly and thus cannot be saved, but rather need to be replaced. Also, when the pathological process cannot be controlled, neurons will continue to die, so patients can only be treated when they have a fully developed cerebellar pathology. Potentiation of cerebellar reserve might be the best approach if sufficient functioning cerebellar tissue remains (e.g., partial traumatic or ischemic destruction of the

cerebellum). Otherwise, replacement of missing neurons by cell grafting to increase cerebellar reserve would be required.

Another issue is distribution of neuropathology—focal or diffuse lesions—which will affect how the transplant potentiates cerebellar reserve or reconstructs the cerebellar structure. In focal cerebellar lesions, complex cerebellar reconstruction, with all cell types in the damaged area, would be needed. Cerebellar organoids can theoretically offer solid pieces to fill "gaps" in the cerebellar structure; however, their synaptic integration is another question. In diffuse pathologies, the basic structure of the cerebellum is preserved so that dispersion of grafted cells through the host cerebellum and their differentiation into a specific neuronal type is the goal. It should be noted that many degenerative diseases of the cerebellum are not limited to the cerebellum but affect also other neural structures and non-neural tissues and organs (e.g., some of the SCAs, Friedreich's ataxia, multiple system atrophy) dysfunction of which contributes to the complex symptomatology. In these cases, treatment of several or all affected structures might be necessary to achieve clinical improvement so that systemic (e.g., intravenous) graft administration would be more applicable. Systemic graft administration has been tested using mesenchymal stem cells (Chang et al. 2011; Li et al. 2018; Oliveira Miranda et al. 2018) that have wider and more general effects. Specific neuronal precursors have relation to specific structures only. Therefore, therapy of multisystem diseases based on substitution of lost cells with specific precursors would probably require in fact several transplantations into several regions.

Age of the patient is another potential factor that could influence the outcome of neurotransplantation therapy as shown in the Parkinson's disease in rats (Collier et al. 1999) as well as in clinical trials (Freed et al. 2001). Tissue from an aged host represents less permissive environment for grafted cells (Collier et al. 1999). One can also speculate that comorbidities, such as brain vessel atherosclerosis reducing oxygen supply, metabolic diseases or changes of immune system functioning can also have impact on tissue. Nevertheless, in aged patients, parkinsonism did not improve regardless of the growth of grafted dopaminergic neurons (Freed et al. 2001). Overall impaired brain functional reserve can limit the repair-response of a specific brain structure by grafting (Freed et al. 2001). Importantly, neural plasticity required for functional integration of the graft substantially decreases with age. Furthermore, ataxic patients should learn to use the graft by training (Rossi and Cattaneo 2002) that could be more intensive and more easily applied in younger patients with better fitness and no comorbidities.

To minimize potential complication, production of cells for neurotransplantation should be in full compliance with good manufacturing practice standardizing cell quality and increasing safety of the therapy. Particularly cell viability is important for good cell survival. Differentiation level, cell type purity, and genetic stability of grafted cells deal with graft-derived tumor risk and should be carefully checked. Chemical and microbial purity are crucial for reduction of biological side effects of residua of culture media components and infection risk respectively.

8 Conclusion

Neurotransplantation is a promising method for therapy of some cerebellar diseases. Nevertheless, many questions remain. So far, there is no definitive proof that transplantation has an acceptable risk-benefit ratio as a routine method, particularly when compared with new less invasive approaches, such as non-invasive cerebellar stimulation, pharmacotherapy, or interventions suppressing pathological allele expression (Zu et al. 2004; Boy et al. 2009; Ilg et al. 2014; Ferrucci et al. 2019; Manto et al. 2021). Unfortunately, recent studies are not systematic, but involve different animal models, with different types of graft, different in vitro culture, storage, and pre-treatment before engrafting, different methods of administration, and follow-up periods. They also have evaluated different parameters (graft histology, biochemical analyses, functional tests, etc.) that are difficult to compare and often give contradictory findings. Therefore, it is still difficult to assess which graft type would be the optimum one for particular cerebellar diseases at their specific stages, and what are the effects of graft preparation and in vitro handling on its survival and functional integration in cerebellar diseases models. A systematic approach, allowing direct comparison of various diseases and various therapeutic approaches, is needed. Future research should also bring details about the mechanisms underlying the graft effect, which could inform selection of the best approach for any particular disease. Considering the variability of cerebellar diseases and pathological states, we need to assess whether neurotransplantation is appropriate, and if so with which type of graft would be the best choice for a specific patient suffering from a particular disease at a particular stage and extent. Systematic, carefully designed controlled studies based on results of pre-clinical experiments should answer these questions specifically for individual types of human cerebellar pathologies.

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Development of Mesenchymal Stem Cells Therapy for the Treatment of Polyglutamine SCA: From Bench to Bedside

Chih-Yuan Ho, Hsiu-Yu Lai, Ling-Mei Wang, and Bing-wen Soong

Abstract This chapter focuses on the clinical application of mesenchymal stem cells (MSCs) for the treatment of spinocerebellar ataxia (SCA). SCA is a progressive neurodegenerative disease with complex etiologies. The therapy of SCA is an unmet medical need and the effective drug for this disease is yet to be developed. Fastly progressing gene- and cell-based therapies offer the alternative therapeutic approaches and may provide more potentials to treat the diseases resulting from multiple pathogenesis pathways. This chapter covers the overview of SCA, MSCs, and their potentials, functional assessment of MSCs on treating SCA in pre-clinical disease models, and potential mechanisms of actions (MoAs) exerted by MSCs. Moreover, clinical trials of utilizing adipose-derived MSCs (ADMSCs) for the treatment of spinocerebellar ataxia type 2 (SCA2) and spinocerebellar ataxia type 3 (SCA3) will be discussed in this chapter. Finally, the opportunities and challenges facing the development of MSCs as the therapeutics for SCA as well as other neurodegenerative diseases will also be discussed in this chapter.

Keywords Cerebellar ataxia · Spinocerebellar ataxia · Stem cell · MSC · Clinical trial · PolyQ · Protein aggregation · Redox balance · Neurodegenerative disease

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_19

Abbreviations

^{18}F-FDG	^{18}F-fluorodeoxyglucose
AD	Alzheimer's disease
ADL	Activities of daily living
ADMSCs	adipose-derived MSCs
ALS	amyotrophic lateral sclerosis
ATMP	Advanced therapy medicinal products
ATP	adenosine triphosphate
ATXN1	ataxin-1 protein
ATXN2	ataxin-2 protein
ATXN3	ataxin-3 gene
ATXN3	ataxin-3 protein
ATXN7	ataxin-7 protein
Aβ	amyloid-β
BBB	blood-brain barrier
BBS	Berg Balance Scale
Bcl-2	B-cell lymphoma 2
BDNF	brain-derived neurotrophic factor
BLA	Biologics License Application
BMMSCs	bone marrow-derived MSCs
CA	cerebellar ataxia
CD	cluster of differentiation
CM	conditioned medium
CNS	central nervous system
CO_2	carbon dioxide
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
EMA	European Medicines Agency
ESCs	embryonic stem cells
EVs	exosomes
FDA	Food and Drug Administration
FGF2	fibroblast growth factor 2
GDNF	glial cell line-derived neurotrophic factor
GFP	green fluorescent protein
GPx	glutathione peroxidase
HD	Huntington's disease
HLA-DR	human leukocyte antigen-DR
ICAM-1	intercellular adhesion molecule 1
ICARS	International Cooperative Ataxia Rating Scale
IL-10	interleukin-10
IL-1β	interleukin-1 beta
iPSCs	induced pluripotent stem cells

LPS	lipopolysaccharide
miRNA	microRNA
MoAs	mechanisms of actions
MSA	multiple system atrophy
MSA-C	multiple system atrophy, cerebellar type
MSCs	mesenchymal stem cells
mtDNA	mitochondrial DNA
NT-3	neurotrophin-3
ODAC	Oncologic Drugs Advisory Committee
PD	Parkinson's disease
PET	positron emission tomography
PGE2	prostaglandin E2
PMDA	Pharmaceuticals and Medical Devices Agency
polyQ	polyglutamine
RMAT	regenerative medicine advanced therapy
RNA	ribonucleic acid
ROS	reactive oxygen species
SARA	Scale for the Assessment and Rating of Ataxia
SCA	spinocerebellar ataxia
SCA1	spinocerebellar ataxia type 1
SCA17	spinocerebellar ataxia type 17
SCA2	spinocerebellar ataxia type 2
SCA3	spinocerebellar ataxia type 3
SCA6	spinocerebellar ataxia type 6
SCA7	spinocerebellar ataxia type 7
SOT	Sensory Organization Testing
SR-aGVHD	steroid-refractory acute graft versus host disease
TNFα	tumor necrosis factor alpha
TSG6	TNFα-stimulated gene-6
UCBMSCs	umbilical cord blood-derived MSCs
UCMSCs	umbilical cord-derived MSCs
VCAM-1	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor

1 Introduction

Spinocerebellar ataxia (SCA) is a progressive neurodegenerative disease with complex etiologies. SCA patients can only have symptomatic treatment since no effective therapeutics are available on the market. Although there have been continuous attempts on developing drugs for treating SCA and other neurodegenerative diseases, many of the drug developments failed at later stage of the trials. At least one

of the possible reasons for the unsuccessful developments may be due to trying to treat the complex diseases with single target and/or pathway.

Cell-based therapy can be viewed as the "living-drug" and "miniature drug factory" which is able to interact with/react to the micro-environment of affected areas and potentially offers "customized" secretomes for treating complex pathological conditions. Mesenchymal stem cells (MSCs), one of the cell-based therapies currently being intensively studied, carry several unique features including homing to injured/affected areas, reacting to the niche and producing cytokines, miRNA containing exosomes, and other molecules. Together, they can then repair, recondition, and promote the regeneration of the injured/affected areas.

This chapter provides an overview of SCA and their pathogenesis, MSCs of various origins and their potentials in neurodegenerative diseases, functional assessment, and mechanisms of actions (MoAs) of MSCs on treating SCA in the pre-clinical disease models. Moreover, phase 1/2 and placebo-controlled phase 2 clinical trials utilizing adipose-derived MSCs (ADMSCs) for the treatment of SCA2 and SCA3 will be reviewed and discussed. At last, the potential and future perspectives of applying allogenic MSCs as off-the-shelf therapeutics in SCA and in other neurodegenerative diseases will also be discussed.

2 Polyglutamine Spinocerebellar Ataxia

Neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple system atrophy (MSA), and SCA that involve progressive decline in neuronal function, often with abnormal deposition of proteins and neuronal cell loss, are one of the most challenging fields in medical research. In the past decades, tremendous efforts have been dedicated to the development of therapies for neurodegenerative diseases and, unfortunately, effective treatments are still lacking. Challenges include the complexity of the mechanisms associated with neuronal loss and pathogenic process being not fully understood that no single molecular pathway was able to modulate disease progression, the absence of efficient biomarkers in most cases, and the hurdle of drug delivery to overcome the blood-brain barrier (BBB) (Volkman and Offen 2017; Akhtar et al. 2021).

Currently, 48 SCAs have been identified (Brooker et al. 2021). In general, the prevalence of SCAs is about 3 in 100,000, however, with a wide regional variation (Ruano et al. 2014). Most SCAs are inherited and the most important unifying feature among SCAs is the pattern of neurodegeneration reflecting on the damage of the cerebellum. Many SCAs have extensive cerebellar atrophy involving all regions of the cerebellum including the molecular, Purkinje cell and granule cell layers, as well as deep cerebellar nuclei (Soong and Morrison 2018). Moreover, some SCAs are characterized by their extracerebellar involvement in the basal ganglion, spinal cord, and peripheral nerves (Paulson et al. 2017). Patients with SCAs not only present various clinical features among different subtypes but also share common

manifestations including gait ataxia and incoordination, nystagmus/visual problems, and dysarthria. Other additional features such as pyramidal/extrapyramidal signs, ophthalmoplegia, and cognitive impairment are specific for some SCAs (Sullivan et al. 2019).

Polyglutamine (polyQ) SCAs, including spinocerebellar ataxia type 1 (SCA1), SCA2, SCA3, spinocerebellar ataxia type 6 (SCA6), spinocerebellar ataxia type 7 (SCA7) and spinocerebellar ataxia type 17 (SCA17), are the most prevalent SCAs that are caused by an extensive CAG (Cytosine, Adenine, Guanine) repeat which encodes for expanded polyQ residues within the mutated proteins. Although clinically heterogenous, common pathological features shared by the polyQ SCAs include neuronal protein aggregation, mitochondrial dysfunction and oxidative stress, autophagy impairment, proteasomal impairment, neuroinflammation, and potential toxic ribonucleic acid (RNA) (McIntosh et al. 2021). The Purkinje cells are known to be the major neuronal population affected during the disease progression. Purkinje cell damage or loss are often observed in the polyQ SCAs. Because the diverse biological functions of the polyQ SCA proteins help to specify the disease pathogenesis of each subtype, the expression of the mutant gene or protein is currently the major target for therapeutic research in this field (Paulson et al. 2017). One of the potential approaches that have attracted intensive interests is the application of stem cell therapy. We next focus on the growing evidence of the potential therapeutic effects of MSCs for neurodegenerative diseases and polyQ SCAs.

3 Derivation, Characterization, and Properties of Mesenchymal Stem Cells

Cell-based therapies have been attracting much attention for their potential to provide promising approach for the treatment of unmet medical needs and are considered the fourth pillar of healthcare. Recently, extensive interest has focused on the application of stem cell-based therapies in tissue repair and disease treatments. Stem cells generally refer to a group of unspecialized cells that possess self-renewal capability and can differentiate into different specialized cells. Among the different types of stem cells, embryonic stem cells (ESCs), which are derived from the inner cell mass of embryos, can generate every tissue in the body and are considered the most powerful stem cell type (Thomson et al. 1998). However, the development and application of ESCs involve destruction of the source embryo which has given rise to intensive controversy and ethical issues (Volarevic et al. 2018). Moreover, directly applying ESCs in in vivo studies also showed formation of teratoma which leads to a major safety hurdle for its clinical applications (Hentze et al. 2009). The famous invention of induced pluripotent stem cells (iPSCs) which are generated from genetically modified fibroblasts by introducing four specific genes has shown the similar characteristics of the ESCs in terms of their differentiation abilities (Takahashi and Yamanaka 2006). iPSCs not only perfectly bypass the controversy of ESCs but also

bring huge potential applications to the field of regenerative medicine. However, due to its pluripotent capability, like ESCs, iPSCs may also develop teratomas when directly applied in in vivo studies (Takahashi and Yamanaka 2006). The tumorigenicity of ESCs and iPSCs has set a huge challenge for their direct application in clinical development. Most research of iPSCs in clinical applications focuses on the use of iPSCs-derived specialized cells, relying on its powerful differentiation ability, for tissue repairing or function regaining by the goal to replace the injured or dead cells (Ye et al. 2013; Doi et al. 2020; Salas et al. 2021).

MSCs are another type of stem cells that attract extensive interests due to their potential therapeutic effects on multiple unmet medical needs. Unlike pluripotent stem cells that could generate or differentiate into all types of cells in the body, MSCs are considered multipotent which are able to differentiate into more than one type of cells. Although MSCs does not bearing the powerful pluripotent differentiation ability, MSCs are proved to possess multiple MoAs that have potential benefits for many disease treatments. The unique characteristics of MSCs make them the most studied and applied stem cell type in clinical development.

MSCs were first described in Friedenstein et al's studies from 1960s to 1970s as a type of non-hematopoietic, osteogenic precursor cells identified from bone marrow (Friedenstein et al. 1968). These osteogenic precursor cells are adherent fibroblast-like cells with the ability to form clonogenic colonies during in vitro culture and can differentiate into osteocytes, chondrocytes, and adipocytes (Friedenstein et al. 1970). Until 1991, Caplan proposed the term "mesenchymal stem cells" to reflect the abilities of this type of cells to differentiate into multiple types of cells that form connective tissue in many organs and illustrated their potential application as a new therapeutic technology of self-cell repair (Caplan 1991). With the growing knowledge of the concept of MSCs in the past 30 years, MSCs have been isolated from not just bone marrow but multiple tissues including adipose tissue (Zuk et al. 2001), umbilical cords (Romanov et al. 2003), Wharton's jelly (McElreavey et al. 1991), amniotic fluid (Tsai et al. 2004), placentae (In't Anker et al. 2004), synovial membranes (De Bari et al. 2001), liver tissue (Herrera et al. 2006), lung tissue (Sabatini et al. 2005), and dermal tissue (Byun et al. 2012). Studies even indicate that MSCs reside in all post-natal organs and tissues in the body (da Silva Meirelles et al. 2006).

With the soaring interest in the field of MSCs research, investigators used various methods to isolate MSCs from a variety of tissues and defined characteristics of MSCs in disparate ways which generated many ambiguities and inconsistencies in the field. To address this problem, in 2006, the International Society for Cellular Therapy proposed the minimum criteria of MSCs: (1) the plastic-adherence ability in in vitro culture, (2) cell surface markers expression being positive for cluster of differentiation(CD) 105, CD73, CD90, and negative for CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and human leukocyte antigen-DR (HLA-DR), and (3) the ability to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro (Dominici et al. 2006), which is currently the most recognized consensus on MSCs definition worldwide.

Although MSCs derived from different tissues share the iconic abilities of self-renewal and differentiation, MSCs derived from different tissues show diverse cell characteristics and tendencies. MSCs from neonatal tissues are characterized by a faster proliferation rate and a greater number of in vitro culture passages until senescence comparing with MSCs from adult tissues (Hass et al. 2011). MSCs from bone marrow (bone-marrow derived MSCs, BMMSCs) and adipose tissue (ADMSCs) have higher colony frequency than umbilical cord blood-derived MSCs (UCBMSCs) (Kern et al. 2006). A donor-matched comparison between BMMSCs and ADMSCs revealed that ADMSCs showed a higher proliferation and adipogenic capacity than BMMSCs, while BMMSCs had a higher osteogenic and chondrogenic capacity compared to ADMSCs. However, the proliferation and differentiation capacities of ADMSCs and BMMSCs varied significantly among the donors (Mohamed-Ahmed et al. 2018).

4 General MoAs of Mesenchymal Stem Cells as a Potential Therapeutic Agent

As mentioned earlier, MSCs are considered having less powerful differentiation ability as ESCs and iPSCs do, which makes MSCs less expected for the goal of replacement of injured or dead cells during tissue repair. However, research has shown MSCs possess multiple MoAs that are critical for disease treatments. Most MSCs-based therapeutic strategies include the use of naïve MSCs, pre-treated MSCs, gene modified MSCs, MSCs and devices or materials combination, or the secretomes of MSCs. All these strategies highly rely on MSCs' general MoAs that will be discussed below.

4.1 Paracrine Effects

Although MSCs are considered heterogenous with remarkable donor-to-donor and intra-population heterogeneity (Phinney 2012) and have distinct characteristics from different source tissues, in general, they are well known by now to possess unique therapeutic advantages through diverse mode of actions. MSCs play an incredible role as a mediator to influence the microenvironments or change the targeted cells' behavior. One of the most important ways is through the release of bioactive molecules or the secretion of extracellular vehicles (together called secretomes) to directly or indirectly affect the microenvironment or cells in the vicinity (paracrine effects) (Caplan and Dennis 2006; Lo Sicco et al. 2017; Liang et al. 2014). Secretomes released by MSCs, including growth factors, cytokines, enzymes, and microRNA (miRNA), have been intensively studied in many disease models and are considered as the major mode of actions of MSCs therapies. Linero et al.

showed that both MSCs and their conditioned medium (CM) induced bone regeneration in surgically created lesions in rabbit's jaws, suggesting MSCs exerted its therapeutic effects mainly by the released paracrine factors (Linero and Chaparro 2014). Convincing evidence showed that MSCs, through its paracrine effects, limited infarct size and improved ventricular function in a myocardial infarction model (Gnecchi et al. 2005, 2006), promoted neovascularization in hindlimb ischemia models (Kinnaird et al. 2004). MSCs act through their paracrine activity to attenuate lung inflammation and fibrosis and cell apoptosis in lipopolysaccharide (LPS)- or bleomycin -induced acute lung injury model (Ionescu et al. 2012; Shen et al. 2015). Numerous secretomes of MSCs have proved their therapeutic potential in protecting neural cells, promoting neuronal survival, spinal cord repair, and functional recovery (Puig-Pijuan et al. 2020; Wilkins et al. 2009; Tsai et al. 2018).

4.2 Immunomodulation

The MSCs-derived secretomes are also highly involved in one of the most important therapeutic properties of MSCs, the immunomodulatory effect. MSCs show strong immunosuppressive properties in mixed lymphocyte reaction assay through secreting soluble molecules (Le Blanc et al. 2003; Klyushnenkova et al. 2005). Today, it has been revealed that MSCs, not just through secretomes but also through cell-to-cell contacts, affect the functions of most immune effector cells (Song et al. 2020; Andrzejewska et al. 2019). For example, MSCs have been shown to inhibit naïve T cells and memory T cells responses to communicate with antigen-presenting cells by upregulating their intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expression (Krampera et al. 2003; Ren et al. 2010); and to inhibit T helper 17 cells differentiation by inducing the production of interleukin-10 (IL-10) and prostaglandin E2 (PGE2) (Ghannam et al. 2010). MSCs not only inhibit apoptosis of B cells but also inhibit cell proliferation by blocking the cell cycle of B cells in G0/G1 phase (Healy et al. 2015; Tabera et al. 2008). MSCs can shift the inflammatory M1 macrophages to an M2 macrophage-like phenotype via PGE2 (Vasandan et al. 2016).

With their unique properties, MSCs are extensively studied in numerous fields of disease that still lack effective treatments for their therapeutic potentials. Table 1 has just listed some examples among many others.

5 Potential Mechanisms of MSCs Therapies in Neurodegenerative Diseases and PolyQ SCAs

Research of MSCs in neurodegenerative diseases has revealed encouraging evidence for their therapeutic potentials through multiple MoAs. One of MSCs' important characteristics that has attracted much attention for their applications in

Table 1 The mechanisms of actions underlying MSC-based therapy

Disease field	Potential MoAs and effects of MSCs treatment
Musculoskeletal diseases (Torres-Torrillas et al. 2019)	MSCs can promote bone regeneration and increase bone strength. MSCs can also inhibit induced osteocyte apoptosis and osteocyte-mediated osteoclastogenesis through the exosome releasing.
Cardiovascular diseases (Guo et al. 2020)	MSCs can protect the myocardium by reducing the level of inflammation, promoting the differentiation of myocardial cells and angiogenesis, increasing apoptosis resistance, and inhibiting fibrosis.
Immune-mediated disorders (Markov et al. 2021)	The mechanism of cell-cell contact in association with trophic factors ranging from cytokine to growth factors plays pivotal roles for MSCs therapies for immune-mediated disorders.
Wound healing (Guillamat-Prats 2021)	MSCs or their derivative products have shown paracrine beneficial effects, regulating inflammation, modifying the fibroblast activation and production of collagen and promoting neovascularization and re-epithelialization.
Liver diseases (Khan et al. 2020)	MSCs regenerative therapy in chronic liver disease has been shown to be effective via their immunomodulation, differentiation, and anti-fibrosis properties.
Kidney diseases (Yun and Lee 2019)	The paracrine effects of MSCs on renal recovery, optimization of the microenvironment for cell survival, and control of inflammatory responses are thought to be related to their interaction with the damaged kidney environment.
Neurodegenerative diseases (Mukai et al. 2021)	MSCs exert their neuroprotective and neurorestorative efficacy via the secretion of neurotrophic factors. Potential MoAs also include immunomodulatory effects, mitochondria recovering, abnormal protein clearance…etc.

neurodegenerative disease treatment is MSCs' neuroprotective feature. Damage or death of neuron cells are the ultimate consequences along with disease progression among many neurodegenerative diseases. MSCs treatment can help maintain or recover more neuron cells in different neurodegenerative disease models (Park and Chang 2020; Park et al. 2014; Chang et al. 2011). Potential mechanisms may include preventing neuron apoptosis (Gu et al. 2015; Yin et al. 2014), enhancing the abnormal protein aggregation clearance (Zheng et al. 2018), fusing with the degenerative neurons (Huda et al. 2016), and repairing the damaged neuronal cells (Liu et al. 2014). In this section, we will discuss some important mechanisms that have been intensively studied and provide observations in our internal investigations.

5.1 MSCs Enhance the Abnormal Protein Aggregation Clearance

The abnormal aggregate of proteins and formation of inclusion bodies are considered cytotoxic and involved in the pathology of many neurodegenerative diseases. Therefore, reducing abnormal protein aggregates by preventing the expression of

the abnormal protein or inducing the clearance of the aggregation becomes a potential therapeutic strategy. Autophagy is one of the cellular pathways to remove misfolded or aggregated proteins, clearing damaged organelles as well as to eliminate intracellular pathogens (Glick et al. 2010). A common observation is that defects at different stage of the autophagy pathway arise in late-onset neurodegenerative diseases (Nixon 2013). Strategy of upregulating autophagy has been shown beneficial to delay disease progression in AD, PD, HD, ALS, and SCA (Spilman et al. 2010; Malagelada et al. 2010; Croce and Yamamoto 2019; Amin et al. 2020; Chen et al. 2020; Chang et al. 2013; Menzies et al. 2010).

Accumulation of intracellular mutant protein aggregates in neurons is the common hallmark of polyglutamine disease. The deposit of the misfolded ataxin-3 protein (ATXN3) is proposed to be the hub of SCA3 pathogenesis, especially the nuclear localization of ATXN3 (Sittler et al. 2018; Reina et al. 2010). Since mice lacking ataxin-3 gene (*ATXN3*) did not show abnormality (Schmitt et al. 2007), many researches developed SCA3 therapies targeting mutant protein clearance through autophagy induction or gene silencing and showed promising results (Onofre et al. 2016; Nascimento-Ferreira et al. 2013; McLoughlin et al. 2018). In Nascimento-Ferreira et al.'s study, overexpression of beclin 1, the initiator of autophagy pathway, in the mouse cerebellum significantly improves the motor function of SCA3 mice (Nascimento-Ferreira et al. 2013). Using antisense oligonucleotide targeting the mutant *ATXN3* gene in mouse brain, the accumulation of polyglutamine-expanded ATXN3 was significantly reduced and the motor impairment and Purkinje neuron defect in SCA3 mice were recovered (McLoughlin et al. 2018). The clearance of misfolded ATXN3 proteins mainly depends on regulating the chaperone system, ubiquitin–proteasome system, and aggregation-autophagy system. These three main misfolded protein clearance systems are found impaired in SCA3, resulting in accumulation and aggregation of misfolded proteins and progressive loss of neurons in SCA3 individuals (Li et al. 2015).

Remarkably, MSCs have been revealed their ability of autophagy modulation in immune and other cells involved in disease pathogenesis (Ceccariglia et al. 2020). One study reported that MSCs enhanced autophagy in the Aβ-treated SH-SY5Y cells, a neuroblastoma cell line, and exerted a neuroprotective effect through reducing the level of amyloid-β (Aβ) in the hippocampus in the AD disease mouse model (Shin et al. 2014). Similar enhanced autophagy was also found in the PD disease model both in vivo and in vitro resulting in significantly reduced α-synuclein in dopaminergic neurons (Park et al. 2014).

Oliveira Miranda et al. showed that repeated administration of BMMSCs promoted neuroprotection and reduced the levels of the soluble fractions of the mutant ATXN3 protein indicating the tendency of diminished aggregation forms of mutant ATXN3 in the SCA3 transgenic mice (Oliveira Miranda et al. 2018). One of our studies also observed similar results in allogeneic ADMSCs treatment in a transgenic SCA3 mice, C57BL/6Cr-Tg(L7-HA/ATXN3*69Q)1Hirai, expressing the N-terminal human ATXN3 with 69 glutamine repeats (ATXN3-69Q) with a hemagglutinin

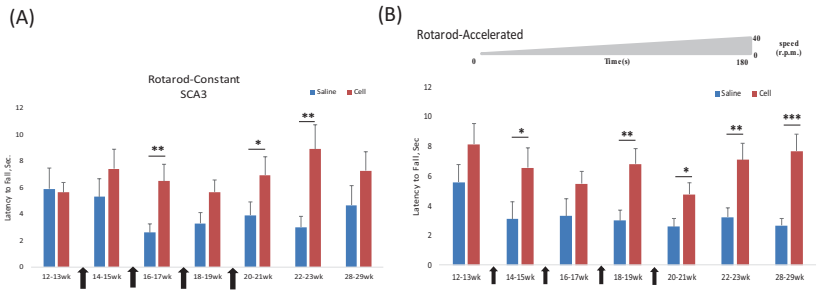

Fig. 1 Rotarod performance of SCA3 mice after receiving 4 infusions of saline or ADMSCs. Timepoint of infusion is arrow-labeled. (**a**) Constant rotarod performance (**b**) Accelerated rotarod performance. (Saline group: $n = 10$, Cell group: $n = 27$, $*p < 0.05$, $**p < 0.005$, $***p < 0.0005$)

driven by the L7 promoter. A significant improvement in rotarod performance was observed after repeated ADMSCs intravenous administration (Fig. 1). The immunohistochemistry analysis of cerebellum from the SCA3 mice showed the nuclear-inclusion form of ATXN3-69Q was decreased in ADMSCs-treated mouse cerebellum (Fig. 2a). Pearson correlation analysis showed the moderate correlation between rotarod performance and toxic ATXN3-69Q aggregate reduction (Fig. 2b). It suggests that the clearance of toxic ATXN3-69Q protein which support neuron survival (unpublished data) may play an important role in ADMSCs treatment in SCA3.

In addition, a SCA3 cell model (Lin et al. 2016), SH-SY5Y ATXN3/Q_{75} cells, a human neuroblastoma cell line SH-SY5Y carrying a doxycycline-inducible green fluorescent protein (GFP) tagged mutant *ATXN3* gene, was adopted to examine the neuroprotective potency of ADMSCs. After introducing doxycycline, the GFP-tagged mutant ATXN3 was expressed (Fig. 3a) and was able to be analyzed by flow cytometry. Our results showed that ADMSCs down-regulated the mutant ATXN3 level of SH-SY5Y ATXN3/Q_{75} cells (Fig. 3b). Treating the neurons with ADMSC-derived CM significantly reduced mutant ATXN3 expressing population, especially the proportion with the middle fluorescence intensity (Fig. 3b), that may be linked with the phenomenon observed in the cerebellum of SCA3 mice receiving ADMSCs. Previous studies proposed that the expanded polyQ stretch may undergo a series of conformational structure transition from monomers, b-sheet-rich monomers, soluble oligomers to insoluble aggregates, and the intermediates including the b-sheet-rich monomer and oligomers appear to be the most toxic species to neurons (Minakawa and Nagai 2021). Both in vivo and in vitro data showed that ADMSCs treatment reduced the level of toxic intermediates through either protein misfolding inhibition or toxic protein clearance system restoration, implicating a potential disease modifying therapy for SCA.

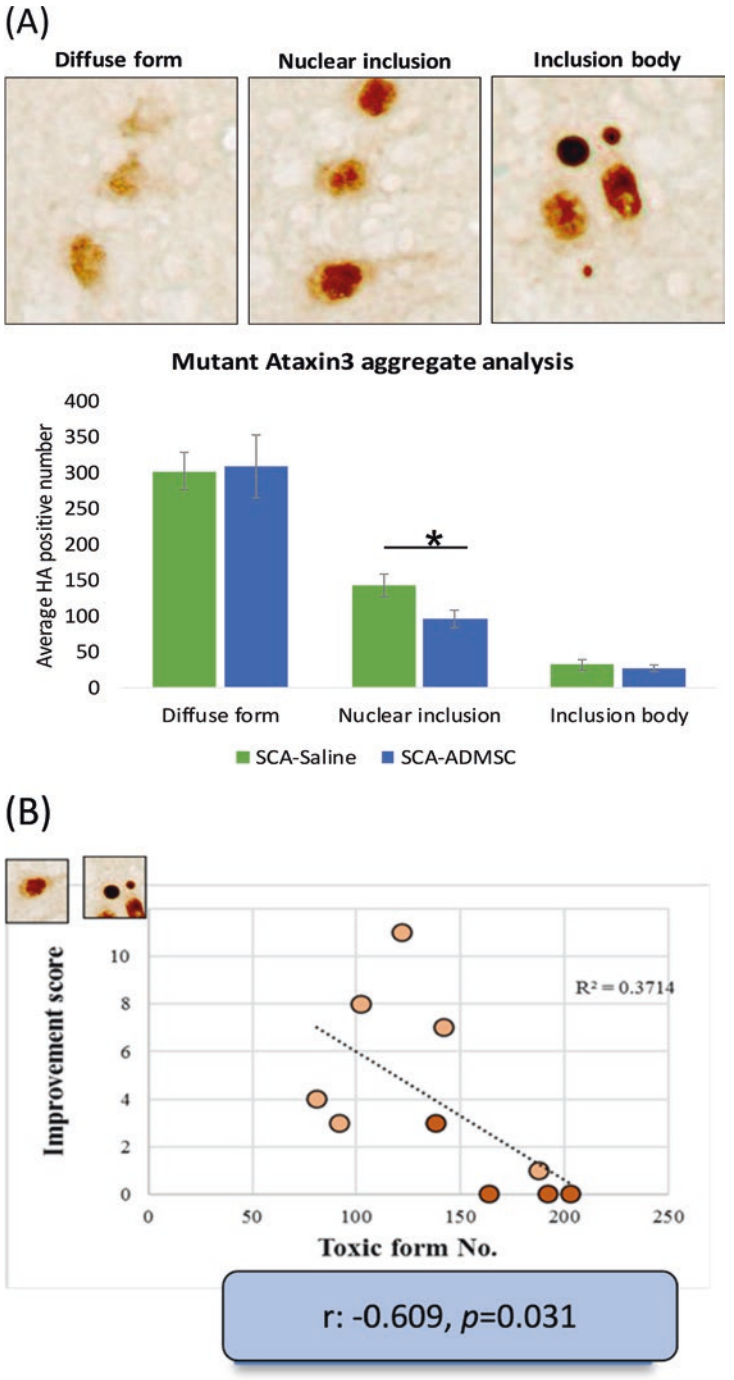

Fig. 2 Mutant ATXN3 aggregates in SCA3 cerebellum. (**a**) three types of human HA-tagged truncated ataxin-3-Q69 (HA-Q69) protein were observed in the tissue sections: diffuse form, nuclear inclusion, and inclusion body. Numbers of different forms of mutant ATXN3 proteins were calculated and only nuclear inclusion ATXN3 was significantly reduced by ADMSCs treatment. (**b**) Pearson correlation analysis showed moderate correlation between rotarod performance and toxic ATXN3 aggregates number (Nuclear inclusion + Inclusion body forms). (Dark dot: SCA-Saline, light dot: SCA-ADMSC)

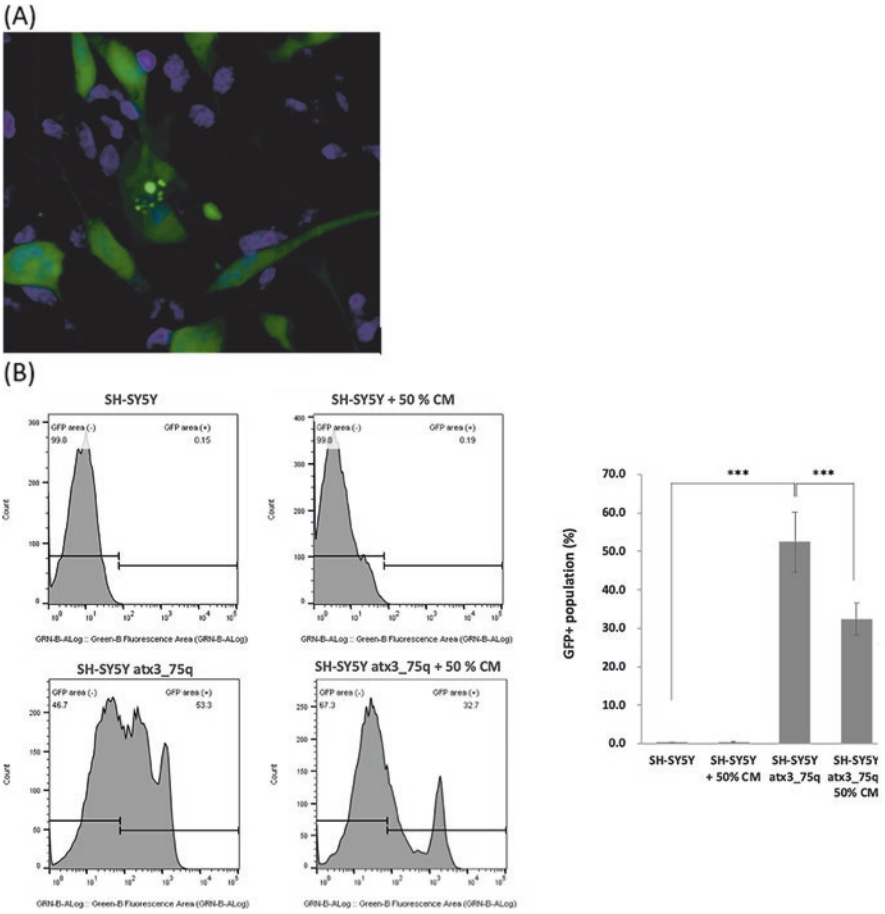

Fig. 3 (**a**) Doxycycline-induced ATXN3/Q75-GFP expression and aggregates formation in SH-SY5Y ATXN3/Q_{75} cell line. (**b**) ADMSC-derived CM reduces the mutant ATXN3 expressing in SH-SY5Y ATXN3/Q_{75} cells. Flow cytometry analysis showed that the GFP positive population, mutant ataxin-3 expressing population, of SH-SY5Y ATXN3/Q_{75} cells is decreased by ~20% after ADMSCs-derived CM treatment

5.2 MSCs Enhance Antioxidant Capability and Exert Anti-apoptosis Effect

Reactive oxygen species (ROS) are recognized as one of the host defending tools by neutrophils to destroy exogeneous bacteria or as cell-signaling molecules to trigger several pathways. However, the excessive production of ROS causes oxidative stress, which has been proposed to be involved in the pathogenesis of many neurodegenerative diseases, including polyQ disease (Gkekas et al. 2021; Angeloni et al. 2020). High level of ROS can cause non-specific modification of proteins, lipids,

and nucleic acids, leading to enzyme inhibition, lipid peroxidation, and deoxyribonucleic acid (DNA) damage, eventually cell death and substantial neuronal cell loss (Di Meo et al. 2016).

The link between aggregated proteins and oxidative stress is well evidenced and ROS can be the cause or the effect of the aggregates (Lévy et al. 2019; Muddapu et al. 2020). Increased ROS can modify molecules on amino acids of protein to cause conformational change which makes proteins prone to form aggregates. In addition, oxidative stress may affect protein degradation process which requires energy for functioning, leading to the accumulation of misfolded proteins. For instance, in the PD patient-derived dopaminergic neurons, oxidative stress could lead to the lysosomal dysfunction and alpha-synuclein accumulation (Burbulla et al. 2017). Reduced antioxidant capacity, that led to increased oxidative stress, also aggravated the severity of PD syndrome in mice (Scudamore and Ciossek 2018). Both heat shock and oxidative stress induced nuclear localization of ATXN3 (Reina et al. 2010). Nevertheless, protein aggregates may also contribute to oxidative stress. It has been reported that the accumulation of misfolded protein aggregates can trigger ROS release during the inflammation (Wyss-Coray and Mucke 2002). In addition, high levels of oxidative stress were generated after application of different forms of α-synuclein to primary rat neurons or human neurons (Cremades et al. 2012). Therefore, oxidative stress and protein misfolding may appear concurrently and aggravate each other to form a vicious cycle, accompanying the inflammation, until neuron cell death.

The mutant proteins in the polyQ disorders also have an association with the increase in oxidative stress and the correlation with the induction of inflammation in disease progression (Gkekas et al. 2021). Increased oxidative stress and abnormalities in the antioxidant system were reported in the serum and fibroblast of the patients with SCA (Pacheco et al. 2013; Cornelius et al. 2017). SCA3 patients had shown a significantly increased peripheral ROS level and decreased glutathione peroxidase (GPx) antioxidant capacity (de Assis et al. 2017). This impaired capability of oxidative stress was found to be associated with disease severity and might contribute to pathogenesis of different SCAs (de Assis et al. 2017; Torres-Ramos et al. 2018; Dennis et al. 2021). Therefore, enhancement of cellular antioxidant capacity and mitigation of oxidative stress may alleviate disease progression (Pohl et al. 2019; Wu et al. 2017; Stucki et al. 2016).

Several studies have shown the antioxidant function of MSCs in treating different diseases. The total antioxidant capacity in cerebrospinal fluid (CSF) of patients with minimally conscious state was increased after BMMSCs transplantations (Jezierska-Wozniak et al. 2020). The GPx1 can be delivered by exosomes (EVs) derived from human umbilical cord MSCs. A single injection of MSC-EVs reduced oxidative stress and cell death of mice with acute liver injury (Yan et al. 2017). After receiving intravenous administration of MSC, the IL-10 knockout mice had a decreased oxidative stress (Jung et al. 2020). Cells from Friedreich's ataxia patients are vulnerable to oxidative stress, when cultured in ADMSCs derived CM, increased cell survival and upregulation of oxidative-stress-related genes were observed (Jones et al. 2012). Exosomes derived from ADMSCs protected motoneuron like

NSC-34 cells, an in vitro model of ALS, from oxidative damage (Bonafede et al. 2016). EVs derived from human Wharton's jelly MSCs were engulfed by hippocampal neurons and protected neurons from oxidative stress and synapse damage induced by Aβ oligomers (Bodart-Santos et al. 2019). Transplantation of MSCs enhanced the redox regulating ability in brain of the AD mice (Yokokawa et al. 2019). All these studies support the potentiality of MSCs in controlling the redox balance in neurodegenerative diseases.

Although ROS can be produced in several organelles, such as endoplasmic reticulum and peroxisome, the mitochondria undergoing oxidative respiration are the major source of ROS (Di Meo et al. 2016). Once the mitochondrial function declines with age or neurodegenerative diseases, it leads to the overproduction of ROS. The overload of the cellular antioxidant defense triggers the mitochondrial dysfunction, energy defect and cell apoptosis, and further contributes to neuronal cell death (Radi et al. 2014; Araujo et al. 2011; Laidou et al. 2020). Studies of MSCs have shown their antioxidant capacity involving the regulation of mitochondrial dynamics and respiratory chain, cytokines secretion and signaling pathways, such as AKT/pAKT and ERK1/2pERK (Baez-Jurado et al. 2019) and their anti-apoptosis capacity indicated by upregulated antiapoptotic B-cell lymphoma 2 (*Bcl-2*) gene expression (Calió et al. 2014). MSCs treatments or their exosomes have been shown to have the neuroprotective effect through the attenuated apoptosis of Purkinje cells in SCAs disease models (You et al. 2020; Atta et al. 2020).

5.3 MSCs Exert Neuroprotective Effect Through Neurotrophic Factor Secretion

Another remarkable therapeutic mechanism of MSCs' for neurodegenerative diseases is their capacity of neural repair. Along with the disease progression, neurons are damaged or periled through a series of negative effects including chronic neuroinflammation (Ising and Heneka 2018), increased oxidative stress (Cenini et al. 2019), cytotoxic aggregation of proteins (Folger and Wang 2021), and dysfunction of mitochondria (Lin and Beal 2006). Mainly relying on their paracrine capability, MSCs have shown exerting neuroprotection through release of multiple neurotrophic factors including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factor 2 (FGF2), and neurotrophin-3 (NT-3) that have been shown to have roles in enhancing adult neurogenesis and neural repair (Volkman and Offen 2017; Joyce et al. 2010; Jones et al. 2010). High levels of BDNF, NT-3, and VEGF were detected in MSCs-treated cerebral ischemia rats that showed functional recovery (Bao et al. 2011). MSCs transplanted into the cerebellum of newborn Lurcher mutant mice with early post-natal death of Purkinje cells in the cerebellum rescued the Purkinje cells and improved motor functions through a trophic mechanism of BDNF, NT-3 and GDNF (Jones et al. 2010). Apart from their innate capability, engineered MSCs to enhance their neurotrophic factor secretion become another

potential strategy for neurodegenerative disease treatments. Treatment by BDNF-releasing MSCs showed an improved outcome in a HD mouse model (Pollock et al. 2016). GDNF-secreting MSCs were observed to provide localized neuroprotection in a PD model (Hoban et al. 2015). BDNF and VEGF co-overexpressing MSCs showed enhanced neuroprotective efficacy in a cerebral ischemia model (Zhou et al. 2017).

5.4 MSCs Modulate the Neuroinflammation Through Their Immunomodulatory Effects

Like their immunomodulatory ability to innate and adaptive immune systems, MSCs have shown critical influence on central nervous system (CNS) inflammation, especially on microglia cells whose activation is the first inflammatory response in the CNS. MSCs, through their sceretomes, inhibited proliferation, secretion of proinflammatory factors, and migration of LPS- activated microglia (Marfia et al. 2016; Garcia-Contreras and Thakor 2021). Not just inhibition of microglia activation but also switch from a proinflammatory state to an anti-inflammatory stage of microglia were also observed in vivo, while MSCs were transplanted in disease models of AD (Ma et al. 2013; Lee et al. 2012), ALS (Vercelli et al. 2008), and cerebellar ataxia (CA) (Nam et al. 2020).

In a study on LPS-induced inflammatory CA mouse model, Nam et al. showed that MSCs inhibited the microglial activation in a dose-dependent manner with significantly inhibited expression of proinflammatory cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNFα) in the cerebellum (Nam et al. 2020). MSCs treatment significantly inhibited the symptoms of ataxia through the anti-inflammatory effect of MSCs-derived TNFα-stimulated gene-6 (TSG-6) and modulatory effect for microglial M2 polarization (Nam et al. 2020). The immunomodulatory effect was also reported in a SCA3 disease model treated with MSCs-derived exosomes that activation of astrocytes was significantly inhibited (You et al. 2020). Since the activation of microglia and astrocytes was observed very early in a SCA1 disease model and the glial activation was closely correlated with disease progression (Cvetanovic et al. 2015), further investigation of MSCs' immunomodulatory effects on neuroinflammation in SCA is important for future strategies on treatment development.

5.5 MSCs Restore Bioenergetic Systems Through Increasing Mitochondria Mass and Enhancing Aerobic Glycolysis

Recently, Wiatr et al. comprehensively investigated the pathogenesis of SCA3 using a humanized *ATXN3* knock-in Ki91 SCA3/MJD mice (Wiatr et al. 2021). In this model, energy metabolism impairment occurs (postnatal day 5, P5) long before the

appearance of motor deficits, suggesting energy deficit may be one of the major factors causing neuronal damages.

Mitochondrion is an essential organelle for adenosine triphosphate (ATP) generation. Previous studies have reported the connections between mitochondrial dysfunction and neurodegenerative diseases that seem to manifest common pathological mechanisms associated with mitochondrial damage. In SCA3, the observed defects related to mitochondria include enhanced oxidative stress, increased mitochondrial DNA (mtDNA) damage, disruption of mitochondria dynamics, collapsed mitochondrial membrane potential, decreased mitochondrial respiration capacity, reduced mitochondrial ATP production, and so on (Elfawy and Das 2019; Harmuth et al. 2018; Hsu et al. 2017; Kristensen et al. 2018; Laço et al. 2012; Raposo et al. 2019). Therefore, restoration of the impaired mitochondria function emerges as a potential therapeutic strategy for neurodegenerative diseases, including SCA3. Many reports have shown that overexpression of selective molecules essential for mitochondrial function or enhancement of mitochondria mass by small molecules can reduce the severity of the polyQ diseases (Sugiura et al. 2011; Ocampo et al. 2010; Ruetenik et al. 2016; Duarte-Silva et al. 2018; de Oliveira et al. 2019). Donation of mitochondria by MSC is proposed to be one of the mechanisms in mitochondria diseases, including neurodegenerative diseases (Gomzikova et al. 2021).

Under normal metabolic circumstances, neuron cells consume glucose and metabolize it completely to carbon dioxide (CO_2). However, during neuronal excitation, glycolysis of glucose to lactate would temporarily exceed the rate of mitochondrial fuel oxidation even when oxygen is abundant, which is typically recognized as the aerobic glycolysis, the Warburg effect (Díaz-García et al. 2017). More and more reports show that the aerobic glycolysis offers several advantages to highly proliferating cells, concerning both bioenergetics and biosynthetic requirements, suggesting it may play an important role in supporting cell growth (DeBerardinis et al. 2008; Lunt and Vander Heiden 2011; Vander Heiden et al. 2009). Aerobic glycolysis is best characterized by the increased glucose uptake and lactate production. It has been reported that enhancement of glucose availability and uptake, as well as increasing glycolytic flux via pharmacological or genetic manipulation of glycolytic enzymes, can be protective in neurodegenerative diseases (Cai et al. 2019; Besson et al. 2015; Hong et al. 2016). In our phase 1/2 clinical study, after administration of ADMSCs, patients with spinocerebellar ataxias had an increment of global glucose metabolism in the brain (Tsai et al. 2017). Further MoAs investigation of ADMSCs in an in-vitro cell model also showed an enhancement of glycolysis in SH-SY5Y cells after CM treatment (unpublished findings), suggesting ADMSCs may exert its therapeutic effects via upregulating aerobic glycolysis, while at the same time rebalancing the redox system in neurodegenerative diseases.

6 MSCs Clinical Studies in PolyQ SCAs

Although enormous MSCs research have been focusing on their therapeutic potentials in neurodegenerative diseases in preclinical stages, numbers of clinical study are still small with most in their early to mid-stages. Even more limited clinical studies exploring on spinocerebellar ataxia have so far been reported mainly to first demonstrate the safety of MSCs as a therapeutic agent in humans. Among these reported studies, although with small patient numbers, MSCs treatment showed some encouraging efficacy results, demonstrating their therapeutic potentials for further clinical investigations. Dongmei et al. reported a study of umbilical cord-derived MSCs (UCMSCs) treatment for 14 SCA patients and 10 MSA, cerebellar type (MSA-C) patients. UCMSCs at a dose of 1×10^6/kg were given through intrathecal injection for four times at weekly intervals. Efficacy measured by the International Cooperative Ataxia Rating Scale (ICARS) and Activities of Daily Living (ADL) scale showed significant improvement in 1 month after treatment. Ten patients remained stable for half a year or longer while 14 patients had regressed to the status prior to the treatment within 3 months averagely (Dongmei et al. 2011). Another UCMSCs study was reported by Jin et al. that 4 consecutive UCMSCs treatments at 1-week intervals were given to 16 SCA patients (including SCA1, SCA2 and SCA3). For the first treatment, 4×10^7 UCMSCs were infused intravenously. For the following 3 treatments, 2×10^7 UCMSCs were infused intravenously and in the meantime, another 2×10^7 UCMSCs were infused intrathecally. Efficacy assessment by ICARS and Berg Balance Scale (BBS) during a 12-month follow-up showed significant improvement at the 3rd and 6th months after treatment compared with the baseline. After 1 year of treatment, 10 patients in ICARS assessment and 7 patients in BBS assessment remained improved (Jin et al. 2013).

We also reported our previous phase 1/2 clinical study of allogenic ADMSCs treatment in 6 SCA3 patients and 1 MSA-C patient. Single intravenous infusion of ADMSCs at the dosage of 7×10^7 cells/20 ml was administered. In the SCA3 patients, the efficacy measured by Scale for the Assessment and Rating of Ataxia (SARA) showed improvement at 6th month after treatment and 4 of the 6 SCA3 patients remained improved or at the same level of SARA score as the baseline at 1 year after treatment. Sensory Organization Test (SOT) also showed an improvement lasting up to 6 months after treatment. In this study, for the first time, we explored the effect of MSCs treatment for SCA3 patients on brain glucose metabolism by ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) analysis. Interestingly, all 6 SCA3 patients showed a trend of improved glucose metabolism from the 3rd month to the 9th month after treatment (Tsai et al. 2017).

Although these three studies were limited with certain restraints, including small patient numbers, mixed types of or non-specific type of SCAs, and no placebo control, they provide preliminary but important information that revealed the potential therapeutic effect of MSCs therapies for SCA. One interesting observation is that, unlike most of the study design in other clinical trials for SCA treatments that the investigational products were taken daily or in a regular base throughout the trial

period (Nishizawa et al. 2020; Zesiewicz et al. 2012; Saute et al. 2014), long-term follow-up periods are generally required by authorities for safety observation in stem cell clinical trials, which were also included in the study design of the three studies, and it gives the opportunities to investigate not just the safety but also the long-term therapeutic effects of MSCs treatments. All three studies presented potential efficacy improvement at the 3rd to the 6th months after the MSCs treatment which indicates sustainable effects of MSCs. This observation may be correlated to MSCs' modulatory properties through their multiple MoAs for neurodegenerative diseases. In our phase 1/2 study, we also compared individual disease progression rate before and during the 1-year study period. After the single infusion of ADMSCs, 4 of 6 SCA3 patients showed a slower disease progression rate during the 1-year study period compared with that of their own natural history (unpublished findings].

To further investigate the potential benefit of MSCs treatment for SCA, we have conducted a randomized, double-blind, and placebo-controlled phase 2 clinical trial with 56 SCA2 and SCA3 patients recruited. Patients received three intravenous infusions of Stemchyaml® at 1-month intervals. To our knowledge, this is currently the largest and the only placebo-controlled clinical trial of MSCs therapy for SCAs. The goals of this trial were to explore: (1) the safety of repeated doses of Stemchyaml®; (2) whether repeated doses can enhance the efficacy observed in the phase 1/2 study, and (3) whether repeated doses will prolong the effective period. This trial is completed and has demonstrated that the application of multiple doses of MSCs is well tolerated and the treatment strategy has the potential benefit to the SCA patients (manuscript in preparation).

7 Opportunities and Challenges of Stem Cell Therapy for SCA

7.1 Multiple Neuroprotective MoAs of MSCs Make Them a Potential Good Therapy for SCAs

Because of the strong belief in therapeutic potential of MSCs, the MSC-based therapy has become an emerging therapeutic approach for the neurodegenerative diseases. In recent years, quite a few publications for preclinical studies (Hernández and García 2021; Schiess et al. 2021; Park et al. 2020; Sykova et al. 2021; Oliveira Miranda 2021) and registered clinical trials (Kim et al. 2021; Venkataramana et al. 2010; Staff et al. 2019) conducted utilizing autologous or allogeneic MSCs derived from various tissue origins to treat diseases including AD, PD, MSA, ALS, and SCAs. The multiple neuroprotective MoAs of MSCs to support the rationale to develop MSCs as the SCAs therapeutics can be best illustrated in Fig. 4. Together, these preclinical studies and clinical trials strongly suggested that MSCs could resolve some clinical unmet needs and may serve as the potential therapeutics for these neurodegenerative diseases which share similar while distinct phenotypes.

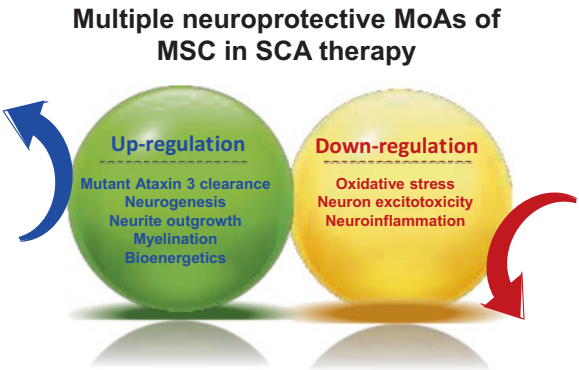

Fig. 4 Proposed MoAs of MSCs in SCA therapy

Although the results from these studies seem to be encouraging, there are challenges facing the development of MSC as the therapeutic drug for SCAs.

7.2 Limited Number and Scale of Clinical Trials Compromise the Outcomes

As of today, there are only three MSC-based clinical trials conducted for SCAs utilizing cells derived from umbilical cord or adipose tissue (Appelt et al. 2021). The most advanced clinical stage registered on the clinicaltrials.gov (keyword: spinocerebellar ataxia and stem cells) is at phase 2 and the numbers of patients for the trials were 20 for phase 1 and 20–60 for phase 2.

With its relatively early clinical stage and the limited patient populations, the cell-based trials often are in small scale with mixed genotypes of SCAs. Because of the diverse pathologies, symptoms, and progression rate among the subtypes of SCAs (Ashizawa et al. 2013; Jacobi et al. 2015; Diallo et al. 2021), the numbers of each SCA recruited in a clinical trial could have impact on the overall results. Therefore, the current observations can provide an overall effect of MSCs therapies for SCAs but are difficult to have statistically sufficient discussion on efficacy for particular genotype of SCA due to the small patient size after breaking down to each subtype of SCA. Well-designed clinical trials with sufficient patient numbers in a single genotype of SCAs are needed to further clarify the therapeutic characteristics of MSCs for SCAs.

7.3 Cell-Based Drug Development Is Unconventional and Regulatory Path Is Still Evolving

The beauty of MSCs therapy also sets challenges for their clinical development as a drug. Unlike the conventional small and large molecule drugs, as the living-drugs, MSCs can interact with/react to the micro-environment and even have the potential

to replicate and/or integrate into host tissues (Caplan and Correa 2011). The diverse potential MoAs give MSCs an unique advantage when it comes to diseases with complex pathogeneses and symptoms. The encouraging results from clinical trials of MSCs treatments for neurodegenerative diseases are more likely synergetic results from the identified potential MoAs. However, challenges for the drug development, apart from supportive clinical outcomes, require establishing comprehensive and specific potency assays for lot release that is correlated to the clinical effectiveness. In the past couple years, the Food and Drug Administration (FDA) has set a new bar for cell characterization, especially for clear critical quality attributes related to MoA and batch release assays for cell therapy developers. The Biologics License Application (BLA) for remestemcel-L, for treatment of pediatric steroid-refractory acute graft-versus-host disease (SR-aGVHD), submitted by Mesoblast Inc., was the most recent example. The FDA did not approve the drug even with the Oncologic Drugs Advisory Committee (ODAC) voted 9 to 1 to recommend the drug for that indication due to the correlation between the in vitro lot release assays and the clinical effectiveness of the product had not been well demonstrated.

This also brings another challenge in SCAs: lacking robust biomarkers for the assessment of disease progression. Currently, the assessment of the disease progression in SCAs mainly relies on scoring systems which must be performed by professionals. However, variations among different raters, the interactions between raters and patients, and various external factors that could affect patient's responses could all contribute to the complexity of the clinical outcome. Therefore, biomarkers are crucial for new drug development. Although imaging biomarkers, CSF, and serum levels of ATXN1, ATXN2, ATXN3, ATXN7 have shown promising results, further studies are needed to determine the utility of these potential biomarkers (Ashizawa et al. 2018)[f] and the relationships with MSCs' potential MoAs.

8 Conclusion

MSCs therapies have shown promising results for neurodegenerative diseases. In SCAs, growing evidence of MSCs treatments has been shown in pre-clinical studies. Early clinical investigations also revealed their tolerable safety and potential long-term therapeutic effects. Further well-designed clinical trials to illustrate the therapeutic benefits and define the MoAs are needed for MSCs to advance in drug development. With the global trend in supporting the advance therapies, we have already seen multiple regulatory supports to encourage and speed up the development of stem cell therapies for unmet medical needs. The examples are: the conditional market approval system of the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan, the regenerative medicine advanced therapy (RMAT) designation of the FDA, and the Advanced therapy medicinal products (ATMP) of the European Medicines Agency (EMA). Although it still faces multiple challenges down the road of its clinical development, we believe that MSCs therapies hold great potentials to benefit patients with neurodegenerative diseases or SCAs soon.

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Cerebello-Spinal tDCS as Rehabilitative Intervention in Neurodegenerative Ataxia

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Abstract Cerebellar ataxias are a heterogenous group of degenerative disorders for which we currently lack effective and disease-modifying interventions. The field of non-invasive brain stimulation has made much progress in the development of specific stimulation protocols to modulate cerebellar excitability and try to restore the physiological activity of the cerebellum in patients with ataxia. In light of limited evidence-based pharmacologic and non-pharmacologic treatment options for patients with ataxia, several different non-invasive brain stimulation protocols have emerged, particularly employing transcranial direct current stimulation (tDCS) techniques. In this chapter, we summarize the most relevant tDCS therapeutic trials and discuss their implications in the care of patients with degenerative ataxias.

Keywords Transcranial direct current stimulation · Non-invasive brain stimulation · Neurodegenerative ataxia · Cerebellar ataxia; therapy.

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1 Introduction

Currently, the vast majority of degenerative ataxias lack effective pharmacologic disease-modifying therapies and there is growing interest in finding innovative therapeutic approaches to improve clinical symptoms in patients with this spectrum of debilitating disorders. A report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology has systematically reviewed the evidence regarding ataxia treatment, with only few studies emerging as promising for the treatment of only a subset of cerebellar ataxias (Zesiewicz et al. 2018). What emerges is that the development of effective therapies may be hampered by the heterogeneity of the cerebellar ataxias and that specific therapeutic approaches may be required for each disease.

In this view, the field of non-invasive brain stimulation has recently gained much attention in the scientific community, in particular because stimulation techniques are non-invasive, provide novel information on cerebellar physiology, may modulate neural plasticity, irrespectively of the underlying disease (Maas et al. 2019a; Grimaldi et al. 2016; Manto and Ben 2008; Grimaldi et al. 2014a; Gandini et al. 2020; Ferrucci et al. 2016; Mitoma and Manto 2018; Benussi et al. 2020), and can be tailored to the needs of specific individuals patients.

In this chapter, we will focus on the principal studies implementing non-invasive brain stimulation techniques, particularly transcranial direct current stimulation (tDCS), in the treatment of patients with genetic and sporadic forms of degenerative ataxias.

2 Transcranial Direct Current Stimulation for the Treatment of Cerebellar Ataxias

2.1 tDCS Techniques

tDCS is a form of non-invasive brain stimulation which modulates neuronal excitability in a polarity-specific manner by delivering prolonged (10–20 minutes) but weak (1–2 mA) currents to brain tissues via electrodes placed on the scalp (Pellicciari and Miniussi 2018). The effects of tDCS are thought to be primarily modulatory, modifying the likelihood of neuronal discharge by shifting membrane polarity. More specifically, anodal tDCS depolarizes the neuron's resting membrane potential and thereby enhances the rate of spontaneous neuronal firing and increases cortical excitability, whereas cathodal tDCS conversely decreases cortical excitability by shifting resting membrane potential toward hyperpolarization, reducing the neuronal firing rate (Nitsche and Paulus 2000). These polarity-dependent changes in cortical excitability are hypothesized to depend on neuroplasticity mechanisms (NMDA-dependent processes) similar to those underlying long-term potentiation (LTP) and long-term depression (LTD) (Liebetanz et al. 2002).

Similarly to what has been observed in the motor cortex, cerebellar tDCS has been shown to modulate cerebellar excitability in a polarity-specific manner, with anodal cerebellar tDCS increasing the excitability of the cerebellar cortex, while cathodal cerebellar tDCS decreasing it (Galea et al. 2009). The change in cerebellar excitability translates in the modulation of the physiological inhibition of the motor cortex by the cerebellum via the cerebello-thalamo-cortical tract, called cerebellar inhibition (CBI), with anodal tDCS increasing the physiological inhibition of the motor cortex, while cathodal tDCS decreasing it (Benussi et al. 2019; Ugawa et al. 1991, 1995, 1997; Werhahn et al. 1996). These polarity-specific effects were confirmed in subsequent behavioral studies performed in healthy controls (Jayaram et al. 2012; Galea et al. 2011; Poortvliet et al. 2018; Cantarero et al. 2015). Animal models have also shown that tDCS may modulate CBI, as observed in humans (Manto et al. 2011; Ben and Manto 2009), and the simple spike activity of Purkinje cells is particularly entrained by alternate current fields, with clear evidence that these neurons represent the primary cell type affected by electrical stimulation thanks to their connectivity and the morphology of their dendritic trees (Asan et al. 2020).

2.2 Clinical Studies

Initial studies evaluated the effects of cerebellar tDCS on neurophysiological measures in ataxic patients. Grimaldi and colleagues observed in nine patients with several types of acquired and degenerative ataxias (one immune-mediated ataxia, one paraneoplastic ataxia, one autosomal recessive spinocerebellar ataxia (SCAR), three spinocerebellar ataxias (SCAs), three idiopathic adult-onset ataxias) a decrease in long latency stretch reflexes after tDCS stimulation with the anode over the right cerebellar hemisphere and the cathode over the contralateral supraorbital area. In this study, short latency stretch responses and coordination tasks in the upper limbs were unaffected (Grimaldi and Manto 2013).

The same group subsequently evaluated the effects of cerebello-cerebro tDCS in two patients with SCA 2. They observed that after 20 minutes of stimulation with the anode over the right cerebellar hemisphere, immediately followed by 20 minutes of stimulation with the anode over the contralateral motor cortex and the cathode over the contralateral supraorbital area, tremor and hypermetria decreased in both patients. These results were accompanied by an improvement in the scale for the assessment and rating of ataxia (SARA) scores (Grimaldi et al. 2014b).

Taking into account that the excitability of the motor cortex is depressed in patients with cerebellar disease (Hore and Flament 1988), Pozzi and collaborators tried to increase motor cortex excitability by placing the anode over the primary motor cortex of the affected side and the cathode over the contralateral primary motor cortex. By applying five sessions of 2 mA stimulation for 20 minutes in three patients with cerebellar ataxia (two with idiopathic late-onset ataxia, one with SCA

6), they observed an improvement in gait, stance, and sitting at the SARA score (Pozzi et al. 2013).

Following these promising results, several randomized, double-blind, sham-controlled clinical trials were performed in larger groups of patients with degenerative ataxia. One of the first was a randomized, double-blind, sham-controlled, crossover study in 19 patients with ataxia (one with SCA 1, five with SCA 2, two with SCA 38, one with Friedreich's ataxia, one with ataxia with oculomotor apraxia type 2, one with fragile-X-associated tremor/ataxia syndrome, six with multiple system atrophy-cerebellar variant (MSA-C), two with idiopathic late-onset ataxia). By applying a single session of 2 mA tDCS with the anode over the cerebellum and the cathode over the right deltoid muscle for 20 minutes, researchers observed a significant improvement in SARA and international cooperative ataxia rating scale (ICARS) scores (particularly in posture, gait, and limb coordination), at the 9-hole peg test and at the 8-meter walking time compared to the sham stimulation. They also observed a significant negative correlation between the improvement in clinical scores and the impairment on activities of daily living. These results were also confirmed when only SCA and MSA-C patients were considered separately (Benussi et al. 2015).

Considering that repeated sessions of stimulation could have cumulative effects on synaptic plasticity (Brunoni et al. 2012), the same group applied the same protocol over two consecutive weeks (Monday–Friday) in a randomized, double-blind, sham-controlled trial in 20 patients with various types of ataxia (five SCA 2, one SCA 14, two SCA 38, one with Friedreich's ataxia, one with ataxia with oculomotor apraxia type 2, one with fragile-X-associated tremor/ataxia syndrome, four with MSA-C, five with idiopathic late-onset ataxia). They observed that a two-weeks' treatment of tDCS with the anode over the cerebellum and the cathode over the right deltoid muscle significantly improved all performance scores (SARA, ICARS, 9-hole peg test, 8-meter walking time) and increased CBI compared to sham stimulation, which were persistent at 3-month follow-up. The improvement in clinical scores significantly correlated with the restoration of CBI. As in the previous study, there was a greater improvement in patients with a reduced impairment in functional scores at baseline (Benussi et al. 2017).

Trying to increase the possible effects of tDCS in patients with ataxia, which frequently have an involvement of the spinal cord, the same group performed a randomized, double-blind, sham-controlled, crossover trial in 21 patients with neurodegenerative ataxia (seven SCA 2, one SCA 14, one SCA 38, one with Friedreich's ataxia, one with ataxia with oculomotor apraxia type 2, six with MSA-C, four with idiopathic late-onset ataxia) by applying a concurrent stimulation with the anode over the cerebellum and the cathode over the spinal lumbar enlargement (2 cm under T11) for two consecutive weeks (Monday – Friday). Also in this case, cerebello-spinal tDCS showed a significant improvement in all performance scores (SARA, ICARS, 9-hole peg test, 8-meter walking time), in motor cortex excitability and CBI compared to sham stimulation (Benussi et al. 2018; Benussi and Borroni 2019).

A following double-blind, auto-matched clinical trial performed in seven patients with ataxia (four with slowly progressive cerebellar ataxia, three with non-progressive cerebellar ataxia) showed a significant improvement of gait parameters and SARA scores after five sessions tDCS with the anode targeting both motor cortices (20 minutes on the left followed by 20 minutes on the right motor cortex) and the cathode over the contralateral supraorbital area (Barretto et al. 2019).

Portaro and colleagues evaluated the effects of anodal and cathodal cerebellar tDCS combined with a robotic gait training in a patient Friedreich's ataxia. The patient was provided with 3 weekly sessions for two months paired with anodal tDCS combined with robotic gait training. After one month, the patient underwent to cathodal tDCS using the same combined tDCS-robot gait training protocol. Both anodal and cathodal tDCS combined with robot gait training demonstrated better improvement in functional motor outcomes on the SARA score and a strengthening of CBI compared to the stand-alone robotic training (Portaro et al. 2019).

An important step forward has been achieved by Pilloni and co-workers, who evaluated the feasibility of long-term at-home treatment with cerebellar tDCS in a patient with cerebellar ataxia. They delivered 60 tDCS sessions, 59 of which were administered remotely, observing an improvement in gait speed and manual dexterity. The applied tDCS montage involved placing the anode over the cerebellum and the cathode over the right shoulder (Pilloni et al. 2019). These findings lay the foundations for the future application of supervised at-home treatment for prolonged periods in patients with ataxia.

On the contrary, two studies performed by the same group did not observe any significant effects of cerebellar tDCS in patients with ataxia. Hulst and co-workers assessed in 20 patients with cerebellar degeneration (five SCA 6, three SCA 14, four with autosomal dominant cerebellar ataxia type III, seven with idiopathic adult-onset ataxia, one with cerebellar degeneration caused by cerebellitis) the effects of approximately 22 minutes of tDCS with the anode over the right cerebellar hemisphere and the cathode over the right buccinator muscle. After 1-week patients received another session of tDCS with the anode over M1 and the cathode over the contralateral supraorbital region. They did not observe any significant effects after cerebellar or M1 stimulation in a standard reaching task with force-field perturbations (Hulst et al. 2017). By applying the same protocol, John and colleagues did not observe significant after-effects of tDCS on grip force control in 14 patients with cerebellar degeneration (two with SCA 6, three SCA 14, five with autosomal dominant cerebellar ataxia type III, three with idiopathic adult-onset ataxia, one with cerebellar degeneration caused by cerebellitis (John et al. 2017).

An aspect which has been frequently neglected in clinical trials in patients with neurodegenerative ataxia is cognitive impairment. While the effects of cerebellar stimulation on cognitive functions have been abundantly studied in healthy controls (Grimaldi et al. 2016), there is a lack of studies assessing the effect on cognition and emotions in patients with cerebellar ataxia. It is now clear that degenerative ataxias frequently have an impairment in several cognitive domains (Teive and Arruda 2009), which have been encompassed in the cerebellar cognitive affective syndrome (CCAS), also referred to as Schmahmann's syndrome, which reflects a constellation

of deficits in executive functions, visuospatial abilities, language and emotion, and which has been attributed to the disruption of pathways connecting the cerebellum with limbic circuits and prefrontal, temporal and parietal association cortices (Schmahmann 2019; Schmahmann et al. 2019). CCAS has been variably described in genetically defined SCAs, in autosomal recessive ataxias, and in MSA-C (Argyropoulos et al. 2020) and the current literature suggests that it should be treated in a specific way depending on the subtype, beyond motor impairment rehabilitation.

Recently, Benussi and colleagues assessed the effects of cerebello-spinal tDCS in 61 patients with cerebellar ataxia also on cognitive dysfunction, in a randomized, double-blind, sham-controlled trial followed by an open-label phase. They observed a significant improvement in the CCAS scale after real tDCS compared to sham tDCS, with an addon-effect after two repeated treatments with real tDCS compared to a single treatment with real tDCS (Benussi et al. 2021). Moreover, they observed an increase in the number of patients with an absent/possible CCAS after real tDCS, who reverted from probable or definite CCAS (Benussi et al. 2021).

In summary, 178 patients have been assessed in 12 published studies using tDCS (see Table 1). A recent meta-analysis, including 5 randomized controlled trials involving a total of 72 participants with cerebellar ataxia, indicated a 26.1% ($p = 0.003$) improvement in ataxia immediately after tDCS with sustained efficacy over months (28.2% improvement after 3 months, $p = 0.04$) when compared with sham stimulation (Chen et al. 2020).

All patients tolerated the interventions without complications and most published studies report beneficial effects in several domains. Several shortcomings however emerge: (1) only a subset of studies used computerized modelling to fully capture the induced electric fields and have not optimized the "'dose" for each participant; (2) protocols are different, including different electrode sizes, electrode shapes, electrode locations, intensity and durations of the interventions; (3) sample sizes are small, with often case reports or case series; (4) few studies are sham controlled and sham tDCS may be problematic (Fonteneau et al. 2019; Neri et al. 2020); (5) blinding is insufficiently assessed; 5) cognitive measures are frequently neglected; (6) outcome measures and reported benefits are variable.

3 Conclusions

Cerebellar ataxias are a very heterogenous group of degenerative disorders for which we currently lack effective and disease-modifying interventions. The field of non-invasive brain stimulation has made much progress in the development of specific stimulation protocols to modulate cerebellar excitability and try to restore the physiological activity of the cerebellum in patients with ataxia but also in other neurodegenerative disorders (Ferrucci et al. 2016). Several different stimulation protocols with tDCS have emerged. A significant limitation of these studies is that methods frequently differ by a considerable degree regarding the area of

Table 1 Studies assessing the effects of tDCS in patients with cerebellar ataxia

Study	Patients	Sham	Blinding	Anode	Cathode	Protocol
Grimaldi and Manto (2013)	9	Yes	Patients	Right cerebellar hemisphere	L supraorbital area	1–2 mA, 20 min
Grimaldi et al. (2014b)	2	Yes	Patients	Right cerebellar hemisphere/Left motor cortex	Contralateral supraorbital area	1 mA, 20 min
Pozzi et al. (2013)	3	Yes	Patients and examiners	Motor cortex affected side	Motor cortex unaffected side	2 mA, 20 min for 5 sessions
Benussi et al. (2015)	19	Yes	Patients and examiners	Cerebellar hemispheres	Right deltoid muscle	2 mA, 20 min
Benussi et al. (2017)	20	Yes	Patients and examiners	Cerebellar hemispheres	Right deltoid muscle	2 mA, 20 min for 10 days
Benussi et al. (2018)	21	Yes	Patients and examiners	Cerebellar hemispheres	Spinal lumbar enlargement	2 mA, 20 min for 10 days
Barretto et al. (2019)	7	Yes	Patients and examiners	Motor cortices	Contralateral supraorbital area	2 mA, 20 min for 5 days
Pilloni et al. (2019)	1	No	Not reported	Cerebellar hemispheres	Right shoulder	2.5 mA, 20 min for 60 days
Hulst et al. (2017)	20	Yes	Patients and examiners	Right cerebellar hemisphere/Motor cortex	Right buccinator muscle/ Contralateral supraorbital region	2 mA, 22 min
John et al. (2017)	14	Yes	Patients and examiners	Right cerebellar hemisphere/Motor cortex	Right buccinator muscle/ Contralateral supraorbital region	2 mA, 22 min
Portaro et al. (2019)	1	No	Not reported	Cerebellar hemispheres	Not reported	Not reported
Benussi et al. (2021)	61	Yes	Patients and examiners	Cerebellar hemispheres	Spinal lumbar enlargement	2 mA, 20 min for 10 days (two treatments vs. one treatment)

stimulation, intensity, and number of sessions, including different clinical or neurophysiological outcome measures. Moreover, the relative infrequency of cerebellar ataxias limits the design of sufficiently powered and homogenous clinical trials. Recently, the rationale and protocol of a randomized, double-blind, sham-controlled clinical trial in patients only with SCA3 have been published and are underway (Maas et al. 2019b).

Nevertheless, several studies both in healthy controls and in patients with ataxia have suggested that the modulation of the cerebellum by non-invasive brain stimulation may enhance postural control, gait, motor coordination, and cognition.

Despite the promising findings, the precise mechanism of action of non-invasive cerebellar stimulation still remains largely unknown. Other than modulating the excitability of Purkinje cells and increasing their activity on the dentato-thalamocortical pathway, several other mechanisms could be involved, such as inactivation or activation of specific cellular processes, including gene expression, protein synthesis, channel pump regulation, and receptor or neurotransmitter modulation (Grimaldi et al. 2016).

Recently, the concept of cerebellar reserve is also emerging, which refers to the capacity of the cerebellum to compensate for tissue loss resulting from several different etiologies. When the inciting event produces acute focal damage (e.g., stroke or trauma), impaired cerebellar function may be compensated for by other cerebellar or extracerebellar areas (i.e., structural cerebellar reserve). On the contrary, when pathological changes compromise cerebellar neuronal integrity gradually leading to cell death (e.g., neurodegenerative ataxias), it is conceivable that the affected area itself can compensate for the slowly evolving cerebellar damage (i.e., functional cerebellar reserve) (Mitoma et al. 2019). It is thus fundamental to apply non-invasive brain stimulation techniques during a phase where cerebellar reserve is preserved and novel tools should be developed to estimate the cerebellar reserve from a functional perspective. Using non-invasive brain stimulation without attempting to estimate cerebellar reserve will end up in conflicting results due to the extreme heterogeneity of cerebellar disorders (Manto et al. 2021).

Several questions remain unanswered and could provide novel targets for future studies. For example, also transcranial alternating current stimulation (tACS) has recently emerged as a new technique to modulate cortical oscillations and entrain brain rhythms in specific frequencies (Antal et al. 2008). By applying cerebellar tACS at a frequency matching the basal firing rate of Purkinje cells (50 Hz), researchers have shown that tACS may modulate CBI and improve the performance of a motor tasks in healthy controls (Naro et al. 2017; Miyaguchi et al. 2018, 2019a, b; Naro et al. 2016). Whether these novel types of stimulation might be effective also in the treatment of cerebellar ataxias is still unknown.

Future studies should try to assess several issues: the inclusion of etiologically homogenous group of patients, perhaps in multicenter studies, defining the optimal timing of follow-up stimulation sessions and the effects of repeated sessions over time, the effects on cognition and emotions, the feasibility of at-home remotely supervised stimulation in larger cohorts, and if these effects may be intensified by

concurrent motor training interventions or pharmacologic therapies. Critically, evaluation of target engagement using imaging or physiologic biomarkers and the assessment of "dose" by using modelling to calculate the induced currents in the brain to define individual stimulation parameters would be essential.

In conclusion, particularly in the light of limited evidence-based pharmacologic and non-pharmacologic treatment options for patients with ataxia, non-invasive brain stimulation techniques with tDCS are potentially promising tools for therapeutic approaches, but further work is needed before it can be broadly offered in the clinical setting.

Acknowledgements This chapter is supported by the Airalzh-AGYR2020 grant issued to Alberto Benussi.

Author Contributions Drafting the manuscript: Alberto Benussi, Valentina Cantoni, Alvaro Pascual-Leone, Barbara Borroni.

Potential Conflicts of Interest Alberto Benussi was partially supported by the Airalzh-AGYR2020; and is listed as an inventor on issued and pending patents on the use of non-invasive brain stimulation for the differential diagnosis of dementia and to increase cognitive functions in patients with neurodegenerative disorders. Alvaro Pascual-Leone was partly supported by the National Institutes of Health (R24AG06142, and P01 AG031720) and the Barcelona Brain Health Initiative (La Caixa and Institute Guttmann); and is a listed as an inventor on several other issued and pending patents on the real-time integration of noninvasive brain stimulation with electroencephalography and magnetic resonance imaging; he is co-founder of Linus Health and TI Solutions AG; serves on the scientific advisory boards for Starlab Neuroscience, Magstim Inc., Radiant Hearts and MedRhythms, and is an Associate Editor for Annals of Neurology. Barbara Borroni is listed as an inventor on issued and pending patents on the use of non-invasive brain stimulation for the differential diagnosis of dementia and to increase cognitive functions in patients with neurodegenerative disorders, and she served on the scientific advisory board for Alector and Wave Life Sciences.

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Cerebellar Transcranial Magnetic Stimulation in Cerebellar Ataxias

Carina França and Rubens Gisbert Cury

Abstract Treatment options for autosomal dominant cerebellar ataxias are still scarce. Transcranial magnetic stimulation (TMS), a neuromodulation technique currently used for the treatment of depression, pain, vascular motor deficit, and posttraumatic stress disorder, can be a symptomatic treatment for ataxic patients. In this chapter, we reviewed current medical literature for the use of cerebellar TMS in spinocerebellar ataxias. Ten articles, including 170 ataxic patients, reported ataxia improvement after cerebellar TMS, with variable, but overall small effect sizes. This procedure appears to be safe since no severe side effect was reported. Additionally, cerebellar TMS can increase cerebellar blood flow, decrease oxidative stress, and decrease inhibition of the cerebellum over the contralateral motor cortex. However optimistic, these results still need to be better investigated in larger, longer, and more homogeneous trials.

Keywords Ataxia · Spinocerebellar ataxia · Transcranial Magnetic Stimulation · Neuromodulation · Cerebellum

1 Introduction

Autosomal dominant spinocerebellar ataxias (SCA) are a genetically inherited group of diseases related to degeneration of the cerebellum and cerebellar pathways (Klockgether et al. 2019). They can be divided into repeat expansions and non-repeat mutations SCAs (Klockgether et al. 2019). The prevalence of SCA, although

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_21

challenging to assess, is estimated to be 2.7 cases per 100,000 individuals (Ruano et al. 2014). All SCAs are invariably progressive, and are responsible for premature disability, impairment in quality of life, and death (Diallo et al. 2018; Schmitz-Hübsch et al. 2010). Currently, there is no treatment capable of slow or cease SCAs evolution, and treatment is limited to symptomatic options. Even so, there is little treatment choices to effectively improve ataxic symptoms.

Pharmacological treatments with positive evidence for SCA treatment include riluzole, valproic acid, lithium carbonate, and varenicline, but evidence of quality of life improvement is scarce (Klockgether et al. 2019). Currently, rehabilitation therapies (physiotherapy, speech, and occupational therapy) remain the best studied treatment options for SCA patients. Although improvement in ataxia scores and in daily function after rehabilitation is well documented, size effects are small, and rehabilitation alone is often not capable of reestablishing functionality (Zesiewicz et al. 2018).

In light of this gap in ataxia treatment, transcranial magnetic stimulation (TMS) has emerged as a possible option treatment for SCA patients (França et al. 2018; Cury et al. 2020). TMS is a non-invasive, safe, and well-tolerated neuromodulation technique that can reach different areas of the nervous system and is capable of long-lasting benefits (Lefaucheur et al. 2020). Currently, TMS is successfully used in the treatment of pain, depression, motor recovery after stroke, Parkinson's disease, spasticity, and posttraumatic stress disorders (Lefaucheur et al. 2020). Since the cerebellum is a neural structure abundantly connected to almost all the central nervous system, involved in the pathogenesis of SCA, endowed with neuromodulation properties, and accessible through non-invasive neuromodulation techniques, it is a possible hub of interest for TMS (Cury et al. 2020).

In this chapter, we reviewed studies using TMS aimed at the cerebellum for the treatment of ataxia. We searched for articles published between January 1, 1996, and October 18, 2021, on Medline (PubMed) using terms "Transcranial Magnetic Stimulation" AND "ataxia." Articles selected for this review should demonstrate clinical results in patients with spinocerebellar ataxia after TMS targeting the cerebellum. We included only articles written in English.

2 Principles of Transcranial Magnetic Stimulation

TMS was introduced by Barker et al. in 1985, following the success of transcranial electric stimulation in modulating the motor cortex, as a less painful way to deliver the electric current to the brain (Barker et al. 1985). Based on the electromagnetic induction principle described in 1831 by Faraday, it can generate up to 2T magnetic field that lasts for 100 μs, and that is able to go unattenuated through scalp structures and then generate an electric field in the brain (Farzan 2014).

The electric field, and consequently the neural structures affected, can be shaped through several variables, such as coil geometry, current orientation, and intensity. Circular coils were the first types of coils used and allow a large, albeit not deep,

area of cortical stimulation (Deng et al. 2013). For a more focal stimulation, figure-of-eight and double-cone coils are preferred, and these are also responsible for deeper stimulation fields (the former more than the latter). However, there is a rapid attenuation of the electric field in depth, which implies that more superficial structures receive most of the electric field (Deng et al. 2013). The stimulation of deeper structures, however, can increase depending on the delivered stimulation intensity, since the intensity of the induced current reduces with the square distance to the stimulation site (Deng et al. 2013). Regarding current orientation, it is known TMS stimulates preferentially axons than cell bodies, and the former are best stimulated by a parallel current. However, additionally to depth, shape, and intensity of stimulation, the effects of TMS must be accounted also for structures distant from the stimulation site, since TMS acts by circuit activation (Lefaucheur 2016). After axonal excitation by TMS, the changes in neuronal membrane spread in both orthodromic and antidromic directions, activating postsynaptic and presynaptic structures, respectively (Lefaucheur 2019). Although the effects of TMS are not exclusively consequence of local effects, but also distant circuit effects, it is important to precisely determine the stimulation target, and for this purpose the use of neuronavigation systems seems to be preferred over skull landmarks (Lefaucheur 2019).

There are several available TMS protocols. Single-pulse TMS (pulses separated by intervals ≥4 s) is largely used to measure neurophysiological variables, such as motor-evoked potentials, which reflect cortical excitability and the integrity of corticospinal pathways (Farzan 2014; Rodríguez-Labrada et al. 2018). Paired-pulse TMS consists of a conditioning stimulus followed by a test stimulus, and both stimuli are separated by an interstimulus interval (Rodríguez-Labrada et al. 2018). In the cerebellum, paired-pulse TMS can be used to measure cerebellar-brain inhibition (CBI) and cerebellar-brain facilitation (CBF) (Ugawa et al. 1995). CBI most likely reflects activation of cerebellar cortex Purkinje cells, which inhibits cerebellar facilitatory output through dentate-thalamic-cortical pathway to the contralateral cerebral motor cortex (Ugawa et al. 1995). The use of TMS in repetitive pulses—repetitive TMS (rTMS)—has a modulatory effect over neural structures possibly through long-term depression and long-term potentiation, and can generate plastic synaptic changes (Chen et al. 1997; Pascual-Leone et al. 1998). High frequency rTMS (≥5 Hz) is considered to be excitatory, while low frequency rTMS (≤1 Hz) is inhibitory. A type of rTMS, theta burst stimulation (TBS), can also be used for neuromodulation, and consists of 50 Hz bursts at 5 Hz delivered continuously (cTBS, considered inhibitory) or intermittently (iTBS, considered excitatory) (Suppa et al. 2016). This notion of inhibitory or excitatory is not always straightforward, since it can vary depending on the stimulation target and the prior state of circuits activation (Lefaucheur 2006; Fitzgerald et al. 2006). As dictated by the Bienenstock–Cooper–Munro model, if postsynaptic activity is high, it is more likely to be depressed; if it is low, it is more likely to be potentiated (Bienenstock et al. 1982). Therefore, the effects of rTMS are more dependent of baseline excitability levels than stimulation frequency (Daskalakis et al. 2006). This is probably one of the reasons why a typical plastic responses and altered excitability modifications to cortical stimulation have been reported in various neuropsychiatric diseases (Ueki et al. 2006; Quartarone

et al. 2003; Pascual-Leone et al. 1996). Additionally, most of the knowledge about rTMS effects is derived from motor cortex studies; the effects after cerebellar rTMS are more limited. The size effect after one rTMS session is usually small, and short-lasting, but its effectiveness can be enhanced if patient is submitted to repeated sessions, especially in consecutive days (Valero-Cabré et al. 2008).

3 The Cerebellum as a Window to the Whole Brain

The cerebellum has emerged as an attractive and promising target for neuromodulation in neurological disorders over the last few years. Because cerebellar areas present several connections with important cortical and subcortical structures, the modulation of these different neuronal networks could potentially treat pathologic neuronal oscillations and thus influence motor and sensory integration (Fig. 1).

Since the cerebral cortex is connected to the cerebellum only by polysynaptic circuits, and hence there are no monosynaptic connections, traditional techniques of anterograde and retrograde tracing cannot explore the topographic relationship between these two structures (Evarts and Thach 1969; Schmahmann and Pandya 1997; Strick 1985). Instead, inferences from deficits after specific lesions, as well as physiological and transneuronal tracing techniques, and functional neuroimaging could be used to investigate correlated areas.

Coherence is a spectral measure of the neural synchrony that can suggest communication between brain areas and can be measured using intrinsic low-frequency functional correlations by functional magnetic resonance imaging (MRI). Buckner et al. used this technique to create a complete functional map of the human cerebellum, and found functional connections between the cerebellum and the entire cerebral cortex, except perhaps primary visual and auditory cortices (Buckner et al. 2011). The cerebellum holds hubs of major functional brain networks, including Somatomotor Network, Default Mode Network, Limbic Network, Frontal Control Network, Ventral Attention Network, and Dorsal Attention Network (Buckner et al. 2011). Despite the previous concept of the cerebellum as a structure purely related to motor control, somatomotor regions occupy only a small portion of the cerebellum; functional connections to cerebral association networks are by far larger (Buckner et al. 2011). Moreover, the cerebellum has at least two complete homotopic maps of all aforementioned cortical networks: one inverted representation in the anterior lobe, and one mirrored upright representation in the posterior lobe. The size of a cerebellar region dedicated to a network is in fact proportionate to its representation in the cerebral cortex, meaning the largest cerebral networks are associated with the greatest representations in the cerebellum (Buckner et al. 2011). This evidence points to a comprehensive cortical representation in the cerebellum.

In addition to cortical areas, several brainstems structures receive cerebellar outputs: pontine reticular nucleus of the tegmentum, basilar pontine nuclei, pontine and medullary reticular formation, inferior olive, red nucleus, periaqueductal gray area, prerubral area, accessory oculomotor nuclei, and superior colliculus

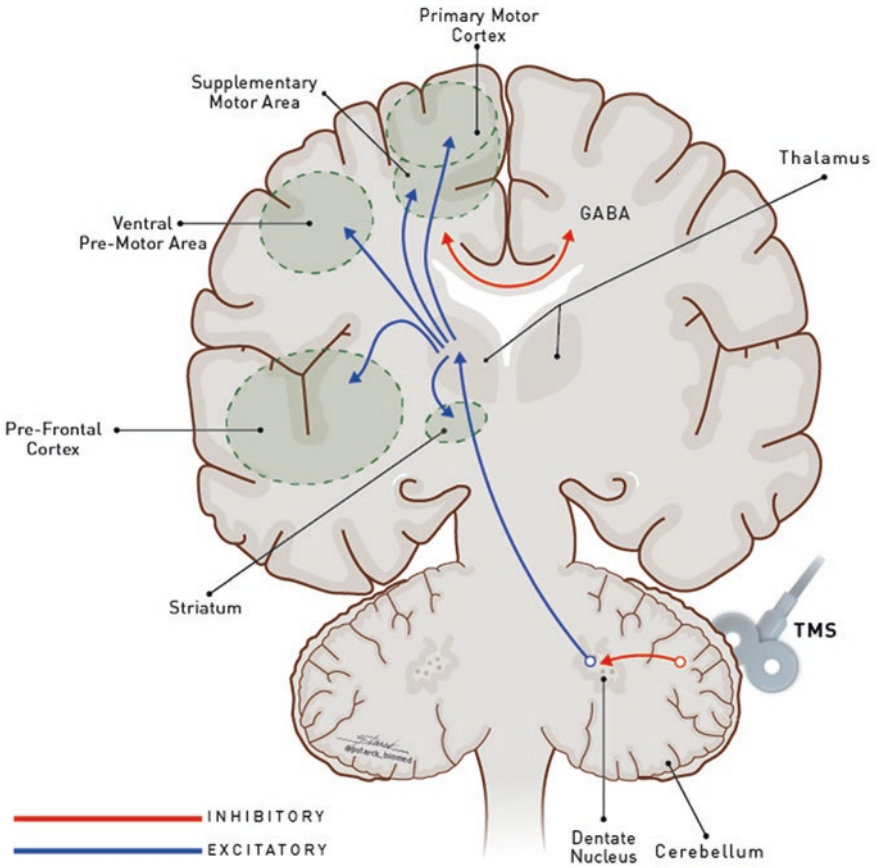

Fig. 1 Schematic representation of cerebellar cortical and subcortical connections. Network model showing cerebellar connections to distant regions. The dentate nucleus receives inhibitory input from Purkinje cells and modulates other brain areas, including contralateral primary motor cortex (facilitatory tonus). There is intracortical inhibition between both motor cortices, which is related to maintaining the integrity of axial, and limbs movements. (Adapted from França et al. (2020))

(Teune et al. 2000). The pontine reticular nucleus of the tegmentum is associated with motor learning (Takeichi et al. 2005), while the inferior olive plays a role not only in motor learning, but also in motor timing (De Zeeuw et al. 1998). Since the red nucleus receives fibers from the dentate nucleus and is connected to both motor cortex and spinal cord, it is associated with motor control, especially postural control (Herter et al. 2015).

The cerebellum is an important source of excitatory input to M1 via the dentato-thalamo-cortical pathway (Fig. 1) and when this input is diminished, there is a reduction in cortical excitability (increase in intracortical inhibition and decrease in intracortical facilitation) (Liepert et al. 2004). Injury in the dentato-thalamo-cortical

pathway reduces excitability in the contralateral cortex (Rispal-Padel et al. 1981), whereas stimulation of the dentate nucleus increases cortical excitability and consequently promotes motor facilitation (Iwata and Ugawa 2005). Therefore, cerebellar neuromodulation techniques can modulate cortical excitability, since the cerebellum is a subcortical structure deputed to plastic mechanisms of motor learning (Ito 2008). However, it is not yet known whether cerebellar stimulation affects the dentate nucleus or Purkinje cells, structures with different roles in the cerebellum-thalamus-cortical activation.

4 Clinical Outcomes

Presently, ten trials evaluated the effects of TMS in spinocerebellar ataxias (Table 1). Overall, 170 patients were evaluated, although additional causes of cerebellar ataxia other than spinocerebellar ataxia were included (multiple system atrophy (MSA-c), post-lesion ataxia, and idiopathic late-onset cerebellar atrophy). Five trials were double-blind and the remaining used open-label or single-blind designs. As for the TMS protocol, six studies used single-pulse TMS, one used low frequency rTMS, one used high frequency rTMS, one used cTBS, and one used iTBS.

All trials reported clinical improvement in ataxia after cerebellar TMS. Shimizu et al., in a preliminary open label study, demonstrated improvement in balance and gait after 21 sessions of single-pulse TMS in 4 SCA patients (Shimizu et al. 1999). Afterward, the same group used a similar protocol on 74 patients with cerebellar ataxia (etiologies not thoroughly described) using a double-blind design, also describing gait and balance improvement. Ihara et al. were the first to describe effects of single-pulse TMS on 20 ataxic patients (mixed etiologies) using a validated ataxia scale, the International Cerebellar Ataxia Rating Scale (ICARS). The most recent single-pulse TMS study included 20 spinocerebellar ataxia patients using a double-blind sham-controlled design and observed statistically significant improvement in the stance sub-score of the Scale for the Assessment and Rating of Ataxia (SARA) (Manor et al. 2019). Dang et al. were the first to use cerebellar rTMS in one SCA6 patient, reporting great improvement in SARA and ICARS (Dang et al. 2019). After 18 months of the last session, this improvement was not only sustained, but increased. The largest rTMS trial included 24 patients (9 SCA3 patients, 8 MSA-c patients, and 7 post-lesion ataxia patients) with a double-blind sham-controlled crossover design and applied five low-frequency rTMS sessions using a deep reaching (double-cone) coil (França et al. 2020). This trial was the first to use neuronavigation to better locate the stimulation target (dentate nucleus contralateral to the most ataxic hemibody) and reported a significant improvement in ataxia using SARA and ICARS comparing active and sham stimulations. Two other

Table 1 Studies investigating cerebellar TMS in spinocerebellar ataxias

Author	(*n*) Population	Target	Protocol	Study design	Main clinical outcomes
Shimizu et al. (1999)	(4) Spinocerebellar degeneration (2 SCA6, 1 SCA1 and 1 SCA7)	Cerebellum (tangentially over the inion, 4 cm to the right, and 4 cm to the left)	Single pulse TMS (1 pulse of 0.1 ms every >5 s, 10 pulses per site, total 30 pulses per session) 21 sessions with 9 cm circular coil at 100% of maximum stimulator output	Open label	Decrease in time and number of steps required for a 10 m walk examination; increase in number of feasible steps in tandem; decrease in total length of tracing body balance.
Shiga et al. (2002)	(74) Spinocerebellar degeneration (cerebellar type × OPCA type)	Cerebellum (over the inion, 4 cm to the left and 4 cm to the right)	Single pulse TMS (1 pulse every 6 s, 10 pulses per site, total 30 pulses per session) 21 sessions with 14 cm circular coil at 250% RMT	Double-blind sham-controlled	Improvement in 10 m time, 10 m steps, tandem steps, and standing capacities, especially in the cerebellar type.
Ihara et al. (2005)	(20) Spinocerebellar degeneration (10 OPCA, 6 CCA, 4 SCA6)	Cerebellum (over the inion, 4 cm to the left and 4 cm to the right)	Single-pulse TMS (1 pulse every 5 s, 10 pulses per site, total 30 pulses per session), 24 sessions with 70 mm figure-of-eight coil at 100% maximum stimulator output.	Single-blind, uncontrolled	Improvement in ataxia (ICARS).

(continued)

Table 1 (continued)

Author	(*n*) Population	Target	Protocol	Study design	Main clinical outcomes
Farzan et al. (2013)	(1) Idiopathic late-onset cerebellar atrophy	Cerebellum (over the inion, 4 cm to the left and 4 cm to the right)	Single pulse TMS (1 pulse every 6 s, 10 pulses per site, total 30 pulses per session) 21 sessions with 14 cm circular coil at 250% RMT	Open label	Improvement of 9% in timed up-and-go test and gait speed. Decrease in stride duration variability and double support time.
Kawamura et al. (2018)	(1) SCA6	Cerebellum (over the inion) and motor cortex (over the vertex)	Cerebellum: Single pulse TMS (20 pulses at 0.5 Hz) 20 sessions with 14 cm circular coil at 50% RMT Motor cortex: single pulse TMS (40 pulses at 0.3 Hz) 20 sessions with 14 cm circular coil at 100% RMT	Open label	Diplopia and limb ataxia improvement after motor cortex stimulation
Dang et al. (2019)	(1) SCA6	Cerebellum (over the inion)	rTMS (10 Hz, 1 s trains, 10 s intertrain interval, 1500 pulses/ session), 20 sessions with figure-of-eight coil at 100% of RMT	Open label	Improvement in ataxia immediately after rTMS (57% in SARA and 61% in ICARS) and 18 months after the last session (82% in SARA and 73% in ICARS)

(continued)

Table 1 (continued)

Author	(*n*) Population	Target	Protocol	Study design	Main clinical outcomes
Manor et al. (2019)	(20) Spinocerebellar ataxia	Cerebellum (over the inion, 4 cm to the left and 4 cm to the right)	Single pulse TMS (1 pulse every 6 s, 10 pulses per site, total 30 pulses per session) 20 sessions with 14 cm circular coil at 100% maximum stimulator output.	Double-blind sham-controlled	Improvement only in stance sub-score of SARA and standing postural sway metrics.
França et al. (2020)	(24) Cerebellar ataxias (9 SCA3; 8 MSA-c; 7 post-lesion ataxia)	Dentate nucleus contralateral to the most affected hemibody (neuronavigated)	rTMS (20 series of 60-s pulses at 1 Hz and inter-train-pulses of 1 s), 5 sessions with double-cone coil at 90% of RMT	Double-blind sham-controlled crossover (≥28 days washout)	Improvement in ataxia (SARA and ICARS)
Lin et al. (2022)	(19) Cerebellar degeneration (13 SCA3; 3 SCA1; 2 SCA6; 1 SCA2)	Right cerebellum (1 cm inferior and 3 cm right to the inion, neuronavigated)	cTBS (3 pulse bursts at 50 Hz repeated every 200 ms for 40 s), 1 session with figure-of-eight coil at 80% of AMT	Double-blind sham-controlled crossover (≥7 days washout)	Improvement in ataxic dysarthria (smaller vocal compensations for pitch perturbations with shorter peak times paralleled by larger cortical P1 and P2 responses and smaller N1 responses)

(continued)

Table 1 (continued)

Author	(*n*) Population	Target	Protocol	Study design	Main clinical outcomes
Sanna et al. (2020)	(6) SCA38	Cerebellum (1 cm inferior and 3 cm left/right to the inion)	iTBS (20 cycles of 2 s of three-pulsed bursts at 50 Hz repeated every 200 ms (5 Hz) repeated every 10 s for a total of 600 pulses), 10 sessions with figure-of-eight coil at 80% of AMT	Double-blind sham-controlled crossover (45 days washout)	Improvement in ataxia (MICARS)

Abbreviations: *AMT* active motor threshold, *CCA* cortical cerebellar atrophy, *cTBS* continuous theta burst stimulation, *ICARS* International Cooperative Ataxia Rating Scale, *iTBS* intermittent theta burst stimulation, *MICARS* Modified International Cooperative Ataxia Rating Scale, *MSA-C* multiple system atrophy cerebellar type, *OPCA* olivopontocerebellar atrophy, *RMT* rest motor threshold, *rTMS* repetitive transcranial magnetic stimulation, *SARA* scale for the assessment and rating of ataxia, *SCA* spinocerebellar ataxia, *TMS* transcranial magnetic stimulation

rTMS trials used TBS protocols and reported improvement in ataxia (iTBS) (Sanna et al. 2020), and more specifically in ataxic dysarthria (cTBS) (Lin et al. 2022).

It is important to highlight that, albeit encouraging, improvements reported in those studies demonstrated highly variable size effects. In the study conducted by Shiga et al., there was 31% decrease in time to walk 10 m, 18% decrease in the number of steps to walk 10 m, and 638% increase in number of Tandem steps achieved, comparing before TMS and after TMS in the active group (Shiga et al. 2002). Ihara et al. reported 5.1 points reduction (improvement) in ICARS score (ranging 0–100) comparing before and after TMS (Ihara et al. 2005). In another study evaluating 20 SCA patients, there was a 3.9 points reduction (improvement) in SARA score (ranging 0–40) after 20 TMS sessions comparing scores from baseline and 1 month follow up (Manor et al. 2019). In the only study using low-frequency rTMS, there was an improvement of 3.3 points in SARA score, and 5 points in ICARS score (França et al. 2020). Finally, in the last published trial, which included 6 SCA38 patients, there was an improvement of 4.4 points in the Modified International Cooperative Ataxia Rating Scale (MICARS, ranging 0–120) (Sanna et al. 2020).

The medical literature up to this point endorses the safety of cerebellar TMS. No clinical study so far reported severe side effects. Some mild side effects included headache or local discomfort, and were all self-limited (França et al. 2020).

5 Neurophysiological and Biochemical Outcomes

In addition to clinical outcomes, several studies also included neurophysiological outcomes, which could help us better understand the pathophysiology behind the clinical efficacy of cerebellar TMS for spinocerebellar ataxias. There is currently evidence pointing to changes of brain blood flow, oxidative stress markers and CBI after cerebellar TMS in ataxic patients.

After 21 sessions of single-pulse TMS over the cerebellum (four patients), single photon emission computed tomography revealed significantly increased blood flow in the cerebellar hemisphere, putamen, and pons, compared to the measures taken before TMS, which may be correlated with the clinical improvement (Shimizu et al. 1999). These findings were then corroborated by a two future clinical trials using single-pulse TMS (Shiga et al. 2002; Ihara et al. 2005). Previous reports showed increase in brain blood flow of normal subjects after TMS both in the stimulated area and associated regions (Siebner et al. 1998).

Ihara et al. evaluated several cerebrospinal fluidbiochemical oxidative stress parameters in 20 patients before and after 24 sessions of single-pulse TMS (Ihara et al. 2005). This is an interesting investigation, since there is evidence pointing to oxidative stress as a pathological mechanism of SCA (Torres-Ramos et al. 2018; Guevara-García et al. 2012; Araujo et al. 2011). Ataxic patients had higher oxidative stress compared to controls, and its levels were inversely correlated with clinical severity (Ihara et al. 2005). This finding suggests decrease of oxidative stress as a possible mechanism underlying the clinical improvement after TMS.

In a single-case study, Farzan et al. examined CBI in a patient with idiopathic late-onset cerebellar ataxia (precise diagnosis unknown). After 21 sessions of single-pulse cerebellar TMS, in addition to the clinical improvement, there was CBI decrease, and this reduction persisted for 6 months after TMS interruption (Farzan et al. 2013). This might suggest reduction of cerebellar tonic inhibition over the cerebral cortex as another mechanism responsible for clinical improvement. Moreover, decrease in the tonic Purkinje cell inhibition may increase vestibular nuclei activity, which could contribute to balance improvement seen in some ataxic patients after TMS (Rub 2002; Shin et al. 2011).

6 Targets and Coils

Location of coil placement, coils shapes, and sizes varied greatly among studies, regardless of the positive clinical outcomes.

The cerebellum is not a homogeneous structure, and is composed of several types of cells, fibers, and nuclei. Some of these components have opposite final effects. For instance, the dentate nucleus is responsible for the excitatory output to the thalamus, but Purkinje cells inhibit the dentate nucleus. Therefore, distinguishing modulation of Purkinje cells and dentate nucleus is paramount, considering

these two structures have opposite roles in cerebellar effects over the motor cortex. However, the determination of the exact brain area being influenced by the induced electric current is a major inherent limitation of non-invasive modulation techniques (Lefaucheur et al. 2020). Most likely, more than one structure is being stimulated simultaneously, and that makes even more difficult to determine which stimulated structure is actually responsible for the final result. This issue is even more complex if we add to the equation the concomitant activation of distant parts of the network, away from the stimulated target (Al-Fatly et al. 2019; Horn et al. 2019).

To comprehend more about this issue, it is important to understand about different coils characteristics. Coils can vary in shapes (circular, figure-of-eight, double-cone, etc.), and sizes (coil diameter). Circular coils are considered superficial coils and can reach a large stimulation area—the larger the coil diameter, the larger the area stimulated. Figure-of-eight and double-cone coils, on the other hand, are deeper reaching coils, and stimulate smaller areas (Deng et al. 2013). The double-cone coil is considered to reach structures as deep as the foot motor cortex (Galhardoni et al. 2019). Since the dentate nucleus lies as deep from the skull surface as the foot motor cortex, it is safe to say double-cone coils are able to reach it (Cury et al. 2015; Hardwick et al. 2014). However, between the dentate nucleus and the skull surface lie Purkinje cells on the cerebellar cortex that could be also modulated by the magnetic field. The electric field diminishes as a function of coil distance; hence, it is possible that Purkinje and dentate nucleus, in addition to other cerebellar structures beneath the coil and its lateral wings, are concurrently modulated at different intensities (Hayward et al. 2007). The insula lies at a similar depth from the scalp as the dentate nucleus (4.5–5.0 cm). Interestingly, TMS insula studies found antinociceptive effects only when using double-cone coils (Ciampi de Andrade et al. 2012; Lenoir et al. 2018). More importantly, this analgesic effect was clinically equivalent to the effect obtained by direct stimulation of the posterior insula using electrodes during electroencephalography in patients with refractory epilepsy (Ciampi de Andrade et al. 2012). These data point to a relatively good specificity and target accuracy when performing TMS with a double-cone coil (Deng et al. 2013). Another study comparing TMS coils found no changes in cerebellar-brain inhibition after cerebellar 1 Hz repetitive transcranial magnetic stimulation with superficial figure-of-eight coil but only with deep-reaching coils (Hardwick et al. 2014). Cury et al. previously reported improvement in the SARA score after cerebellar rTMS using double-cone coil in one post-lesion ataxic patient, and after this same patient received a dentate nucleus Deep Brain Stimulation implant, the improvement in SARA was identical, which would argue in favor of the dentate nucleus as responsible for the clinical improvement (Cury et al. 2015; Teixeira et al. 2015). Currently, we do not have an answer for this conundrum. The most probable explanation might involve effects from multiple structures acting in resonance.

Another possible mechanism is derived from studies of neuromodulation for Parkinson's disease (PD). In PD patients, it is well known that beta oscillations (13–30 Hz) are greatly enhanced, and its presence is correlated with parkinsonian symptoms (rigidity and bradykinesia) (Little and Brown 2014). Levodopa therapy and high frequency Deep Brain Stimulation can reduce beta oscillations, and

improve parkinsonian symptoms. If there is a specific diseased cerebellar activity that correlates with ataxic symptoms, this activity could be disrupted by TMS, and this disruption could be responsible for the clinical benefit observed across studies. However, more studies are required to verify this hypothesis.

7 Little Brain, Big Expectations: A Glimpse into the Future

Albeit homogeneously reporting clinical improvement, studies investigating cerebellar TMS in SCA patients are wildly heterogeneous regarding coil type, frequency, intensity, location, number of sessions, follow-up, evaluation tools, and additional outcomes (França et al. 2018). It is therefore important to corroborate these finding with larger studies using the same stimulation parameters, and with longer follow-ups. Moreover, the fact that all studies reported positive clinical outcomes, but chose different types of TMS, makes us wonder what would be the best TMS setting. More likely, there is not a single answer for all SCA patients since there are different pathological mechanisms depending on the SCA type (Klockgether et al. 2019). In that line, studies should try to include homogeneous populations—a single type of SCA—or post-hoc analysis considering the molecular diagnosis. The main difficulty lies in the heterogeneous rarity of SCAs. SCA3, for instance, is one of the most common SCA, while SCA38 can only be observed in three family clusters (Klockgether et al. 2019; Sanna et al. 2020; Gazulla et al. 2020; Borroni et al. 2016). With this low prevalence in mind, and considering TMS effects are time-limited, multicentric crossover trials seem to be the best path.

Important progress should also be directed to better understand cerebral activity in SCAs. In Parkinson's disease, it is now known that the excess of beta oscillation is correlated with rigidity and bradykinesia (Kühn et al. 2009). Therefore, it is considered an oscillopathy. Both dopaminergic medications and Deep Brain Stimulation therapy can overwrite this pathological activity, and improve symptoms. In ataxia there could be a similar diseased-dominant frequency correlated with the symptoms, and this could potentially be overwritten by neuromodulation. Neurophysiological studies, and, in the future, studies using closed-loop DBS could aid in this matter (Arlotti et al. 2016).

Effect size reported in trials of cerebellar TMS studies for SCA are variable, and most are small. This is a constant in SCA clinical trials so far, regardless of the treatment approach. Romano et al. tested the efficacy of riluzole versus placebo in 55 patients (different types of SCAs and Friedreich ataxia) and found a decrease in SARA scores by 1.02 points in patients (Romano et al. 2015). Another group studied valproic acid in a smaller sample of 12 SCA3 patients and reported a 2.05-point decrease in SARA scores (Lei et al. 2016). However, although an one point decrease in SARA, a scale with a 40-point range, may seem small, it was considered to be clinically relevant in previous studies (Klockgether et al. 2019; Schmitz-Hübsch et al. 2010). Clinical trials combining treatment options (for instance, neuromodulation and physiotherapy) seem to be the natural next step, so we can best evaluate if the combination of treatments could enhance effect size.

An important shortcoming of most trials so far is the absence of quality-of-life measures. It is impossible to understand the degree of impact a certain scale improvement has in a patient's life if quality of life is not evaluated. The only study so far in which quality of life was assessed did not report significant improvement after TMS (França et al. 2020). However, follow-up might have been insufficient to detect real improve in day-to-day activities. It is vital that future trials include quality of life in its outcomes (perhaps as main outcome) and have appropriate follow-ups.

To date, there are no studies comparing TMS to other non-invasive neuromodulation techniques in ataxic patients. In theory, TMS induces a more focal and deeper electric field when compared to transcranial direct current stimulation (tDCS), and can activate specific neural circuits (Di Lazzaro and Rothwell 2014). Comparative studies for pain and upper limb recovery after stroke showed superiority of TMS over tDCS in chosen protocols (Attal et al. 2016; Doris Miu et al. 2020). However, tDCS is a simpler technique, and can be used at bedside, which widens its use possibilities. Studies comparing TMS to tDCS in ataxic patients are needed.

Another gap that needs to be filled is the selection of good responders. Almost all trials up to this point included patients with different ataxia types. Shiga et al. divided patients into two groups—cerebellar type (hereditary and sporadic cerebellar atrophy, including SCA6), and olivopontocerebellar atrophy (OPCA; MSA, SCA1, SCA3)—and reported better outcomes in patients from the OPCA group (Shiga et al. 2002). França et al. included patients with MSA, SCA3, and post-lesion ataxia, and found best improvement in MSA patients (França et al. 2020). Despite these post-hoc analysis results, there is still a paucity of information regarding differences between good and bad responders. Does cerebellar connectivity influence clinical response? Or is it a matter or cerebellar atrophy? A previous study found no correlation between cerebellar volume and clinical outcome after low frequency rTMS (França et al. 2020). Is it possible that integrity of superior cerebellar peduncle (cerebellar efferent pathway) plays a role in clinical improvement after cerebellar TMS? Or are there biochemical differences responsible for the different outcomes? Manto et al. discussed the concept of cerebellar reserve—how much of the cerebellum cells and synapses are still intact—as a way a measure the potential of improvement after cerebellar non-invasive modulation (Manto et al. 2021). With that in mind, perhaps there are no good or bad responders, but good or bad treatment timings. Many questions still need to be answered before we can understand which patient profile could benefit the most.

8 Conclusions

There is evidence to suggest cerebellar TMS is safe and can reduce ataxic symptoms in SCA patients. Additional evidence suggests it can also increase cerebellar brain blood flow, decrease brain oxidative stress, and decrease CBI. Although encouraging, these results should be further explored in larger, more homogeneous trials, and

trials with longer follow-ups. The pathophysiological mechanism of this improvement also should be better explored, as well as characterization of good and bad responders.

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Physical Therapy in Cerebellar Ataxia

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Abstract Given the essential role of the cerebellum in coordinating and refining motor behavior, people with cerebellar disease exhibit significant disability in functional performance. A hallmark of cerebellar damage is ataxia, uncoordinated movement, that is highly variable. Rehabilitation is the primary means of addressing ataxia; however, impaired motor learning makes rehabilitation difficult. Literature guiding physical therapy for ataxia is gradually growing to provide evidence that exercise training is able to modify performance. This chapter describes the features of limb ataxia, postural imbalance, and gait deviations seen in people with cerebellar damage. Compensatory and intervention strategies that clinicians can use in recommending and implementing treatment for their patients are identified. Particular attention is given to balance training for improving gait as this is a primary impairment contributing to a person's quality of life. Future directions to improve rehabilitation for ataxia are considered.

Keywords Ataxia · Imbalance · Incoordination · Exercise · Gait · Rehabilitation · Adaptation · Reinforcement

Physical therapy is not just a means for teaching solutions for maintaining walking with adaptive equipment, like the use of a cane or walker. Therapeutic interventions can offer real physical benefits, but can we perfect those solutions? In rehabilitation, we are able to specifically address weakness and deconditioning through strength and endurance training, though these problems are not primary problems of ataxia. In order to treat ataxia what we need to do is restore balance and stability through learning of new motor patterns which depends on neural plasticity and motor learning ability.

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_22

1 What Is Ataxia

Cerebellar damage may result in oculomotor disorders, slurred and dysarthric speech, uncoordinated limb movements, postural and gait disturbances (Sanger et al. 2006; Bastian 1997; Trouillas et al. 1997). Ataxia is simply described as disordered movement (Bastian 1997). Qualitatively, one thinks of limb ataxia as showing an inability to reach a target and taking an abnormal path getting there. Gait ataxia looks unbalanced, there is veering and often staggering during walking (Ilg and Timmann 2013; Morton and Bastian 2007; Thach and Bastian 2004). Clinically, patients experience clumsiness, imbalance, veering gait, fatigue, and falls. In addition to what ataxia looks like, importantly, with cerebellar damage there is also a deficit in (re)learning a movement pattern (Bastian 2008).

2 What Is Not Ataxia

Cerebellar ataxia does not include weakness or sensory loss, though many disease processes may also include these impairments. Unlike cerebellar ataxia, uncoordinated movement from proprioceptive impairment can be largely compensated for with vision. This may be observed in the heel to shin test where watching one's movement improves accuracy and control for the person with damage to the dorsal column but not for one with cerebellar disease. Ataxia is not associated with abnormal movement from involuntary movements, spasticity, dystonia, or resting tremor (Sanger et al. 2006).

3 Limb Ataxia

Clinically, the symptoms of cerebellar damage are measured by rating scales (e.g., International Classification of Ataxia Rating Scale (ICARS), Scale for the Assessment and Rating of Ataxia (SARA), and Action Research Arm Test (ARAT)) and in the laboratory with kinematics (Trouillas et al. 1997; Ilg et al. 2009; Reoli et al. 2021a). Two impairments of movement observed in limb ataxia are (1) dysmetria, the inability to scale movements resulting in hypermetria and hypometria, and (2) dyssynergia, the inability to coordinate multi-joint movements (Bastian 1997; Bastian et al. 2000). Bastian et al. (1996) demonstrated that when reaching a target, people with cerebellar disease showed a more curved and variable path along with overshooting and undershooting. Moving more slowly than preferred reduced these movement faults whereas moving quickly worsened these features of ataxic reaching. Accuracy was also improved in reaching a target when movement was constrained to only the elbow compared to when both the elbow and the shoulder moved freely. Similar patterns of dysmetria and dyssynergia are observed in leg

movement with heel to shin testing and activities such as walking in tandem. These movement faults are explained by abnormal control of limb dynamics. The inability to scale muscle torques to counter the interaction torques of the movement leads to incoordination of the elbow and shoulder movement and missing the target. Bhanpuri et al. modeled the limb dynamics of people with ataxia in reaching and provides an example of how abnormal limb dynamics lead to impaired feedforward control: In the case of hypometria, a person's internal model of their limb dynamics may be estimated to have greater inertia than the actual presentation so that on initial limb movement the velocity is faster than is required (Bhanpuri et al. 2014). As the limb approaches the target, visual feedback alerts the system that the limb is closer to the target than expected and is on course to pass the target and so makes a correction. However, the new output generated is again too fast which overcorrects the movement in the opposite direction and yields undershooting of the target as it is based on the original assumption that the limb has increased inertia. Predictive control of limb dynamics is necessary to be able to predict interaction torques to counter or exploit them. The cerebellum is important for smoothness, consistency, and accuracy of multi-joint limb movements.

4 Postural and Gait Ataxia

Cerebellar ataxia also may present as imbalance in both sitting and standing. Imbalance can include trunk titubation, difficulty maintaining balance with limb movements, increased postural sway, and abnormal responses to perturbation (Timmann and Horak 1998; Horak and Diener 1994; Ilg and Timmann 2013). Horak and Deiner (1994) showed that people with ataxia showed hypermetric postural responses to perturbation. Increased postural sway varies based on lesion location (Dichgans and Mauritz 1983). Cortical cerebellar atrophy tends to cause greater excursions in sway in the anterior-posterior direction. In contrast, vestibulocerebellar lesions cause increased sway that is nonspecific in direction (Dichgans and Mauritz 1983).

Ataxic gait deviations include deficits of staggering gait, instability, difficulty with stopping and turning, irregular foot placement both in length and width and irregular step timing. There is also abnormal inter-limb coordination (Earhart and Bastian 2001). Compensatory gait characteristics include a wide base of support, high stepping, decomposition and slowed walking with reduced joint excursions, stride lengths and percent swing times (Palliyath et al. 1998; Morton and Bastian 2004; Hallett and Massaquoi 1993; Earhart and Bastian 2001). The Dynamic Gait Index is a clinical measure that demonstrates the impact of gait ataxia in impaired function, increased risk for falls, and reduced quality of life (Keller and Bastian 2014; Reoli et al. 2021a, b).

Morton and Bastian (2003) asked the question: how much do leg incoordination and imbalance contribute to gait ataxia? In 20 healthy controls and 20 people with ataxia, dynamic weight shifting (standing balance), leg placement to a target (limb

coordination), and fast walking velocity were assessed. The hypothesis was that balance and leg placement deficits would cause different gait abnormalities observed in people with cerebellar damage. There was a mix of presentation in the participants with ataxia, some had larger impairments in leg placement or balance and some in both or neither. The data showed that people with primarily leg placement deficits walked relatively normally whereas people with primarily balance deficits showed the most features of gait ataxia and walked poorly. Regression modelling indicated that people with imbalance with or without leg placement deficits walked slower than those without imbalance. These results were consistent with animal literature, which suggests that cerebellar control of balance and gait are interrelated and dissociable from cerebellar control of voluntary, visually guided movements (Ilg et al. 2009; Morton and Bastian 2003).

5 Balance Training for Gait Ataxia

Balance training is an informed choice for rehabilitation for people with ataxia given that ataxic gait is highly related to imbalance and the available studies in the literature using rehabilitation interventions that included or focused on balance have improved walking (Barbuto et al. 2020). A systematic review of the literature by Barbuto found 14 studies having a component of various balance training exercises used to address symptoms of cerebellar disease. The multi-faceted approach to rehabilitation is more similar to general practice where a clinician addresses multiple aspects of patient's performance at a time. With this mixed intervention approach, there might be greater potential to show a decrease in ataxia severity from rating scales that also assess limb ataxia in addition to balance and gait. However, the mixed approach limits our understanding of the impact of balance training on gait function specifically.

To highlight the effect of balance on gait function, we discuss the details of our study, Keller and Bastaib (2014), that used a home-based balance training intervention to improve walking in people with degenerative cerebellar ataxia. Given that balance impairment impacts walking more than voluntary leg control, the program focused on challenging balance activities rather than gait training. Home practice consisted of both sitting and standing exercises, making the intervention accessible to those with a range of disability from independent ambulation to those requiring hand support. Participants were instructed to complete 20 minutes of exercises, 4–6 days for 6 weeks with a mid-point 3-week check-in for program modification as needed. Fourteen individuals completed the training and pre-post clinical and laboratory assessments. Participants logged their days practiced as well as how confident they felt completing the exercises. Participants complied with the training having an average completing 23 (SD = 6) days of practice and a confidence at maintaining their balance in individual exercises of 47% (SD = 20). There was a range of intensity by individual participants such as an individual exercising a large

duration (30 days) with a low level of challenge (13%) to ones with smaller duration (14 days) with a high level of challenge (70%).

Home-based balance training improved test results that were not directly part of the training. Laboratory measures of walking showed both velocity and stride length increasing and double support time decreasing significantly. The Dynamic Gait Index (DGI) indicated improved clinical activity level walking performance. These gait parameters improved over training and were retained 4 weeks post-training. In comparison, the Timed Up and Go, which is used as a measure of gait and balance, showed improvement only over the training period that was not retained. Interestingly, the laboratory measures of balance, static sway amplitude with feet apart eyes open or closed, while showing a trend in improvement did not show significance. There was also no significant change in disability as measured by the ICARS.

A key finding regarding dose was that the level of challenge of the exercises most impacted improvement in walking speed over age, disease severity, or duration of exercise. Intensity of the program was determined both by duration (days of practice over 6 weeks) and degree of challenge. A modified scale to rate challenge (Fig. 1) was given to the participants that was a combination of the Modified Borg Perceived Rating of Exertion and the Activities Balance Confidence Scale. The scale is useful both as a measure of where to start a program and also when to make changes in the intensity in order to progress performance. Given the risk of falls for this population (Schniepp et al. 2022), the exercises need to start with what the person is able to do both independently and safely. Therefore, structuring the environment for safe performance and having a caregiver provide standby assistance are key for home

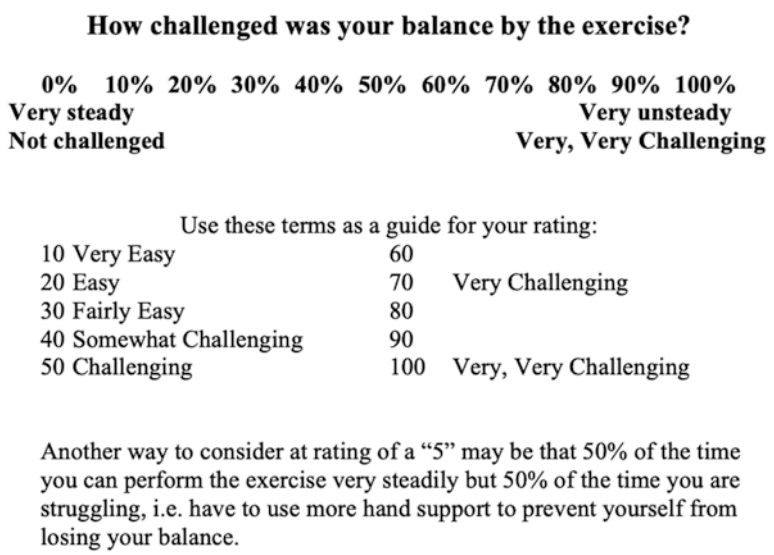

Fig. 1 Effort rating handout for determining balance challenge

practice to be successful. This may be an indication for when in-person therapy is necessary to ensure safety if lack of support or reduced executive function limits a person's ability to perform exercises at a challenging level. A hybrid option using the coaching model with telehealth could also be an intermediate option that could meet the needs of those who need more guidance or for whom access to skilled professionals is lacking (Manto et al. 2020). It is unclear exactly how much challenge is needed, but with limited cerebellar function it may be that the system needs to be pushed harder to make the desired changes.

Improved walking performance from rehabilitative training has been demonstrated in both home training and intensive outpatient therapy (Keller and Bastian 2014; Ilg et al. 2009). Ilg et al. (2009) showed after a 4-week intensive coordinative (balance, body coordination, and stretching exercise) outpatient program, people with ataxia improved walking velocity, dynamic balance, and disability. This study suggested that the subgroup of patients who started from a more severe state and presented with greater sensory than cerebellar ataxia had limited benefits from training over those with only cerebellar ataxia (Ilg et al. 2009). The program also included gait and coordination training that improved multijoint coordination and contributed to the improved disability ratings from the SARA, as Barbuto has postulated (Barbuto et al. 2020). The two studies also indicate that the home program mostly likely needs to be continued and modified as people progress in order to continue to maintain benefits.

6 Motor Learning in Cerebellar Disease

How do people with cerebellar disease respond to rehabilitation, given that motor learning that is necessary for skill modification is impaired? Adaptation, error-based learning, is moderated by the cerebellum in motor control. Many movement types including reaching, posture, and gait control use trial and error practice for learning (Horak and Diener 1994; Shadmehr and Mussa-Ivaldi 1994; Reisman et al. 2005). This learning mechanism develops during childhood and is not fully functional until around 12 years of age (Vasudevan et al. 2011). Cerebellar damage impairs the ability to predict and update the motor commands for motor skills when perturbed in novel situations (Morton and Bastian 2006; Reisman et al. 2007). Instead people with cerebellar disease report how taxing movement is as they have to think and watch what they do and cannot rely on an automatic process to accomplish a new task so that they do better walking if they have limited distraction (Lang and Bastian 2002). However, people with cerebellar disease do respond to rehabilitation. Reinforcement learning may be one way to intervene. Reinforcement learning provides a binary feedback of success or failure. The real-time information about the movement is masked and the process does not rely on error at all. Therrien et al. have shown that people with cerebellar disease are able to learn and retain a new movement task with binary reinforcement (Therrien et al. 2016). Additionally, using virtual reality, Therrien et al. have demonstrated that reinforcement feedback can be used to reduce corrective movements and smooth the path of reaching (Therrien

et al. 2021). This learning occurs as a result of exploration of different movements and selecting patterns that give success. Results suggest the benefits are different from massed practice or simply reduced visual control (Hasson et al. 2015). Clinically, in balance training to improve gait in ataxia, reinforcement learning might be used when the person with ataxia considers using (negative) or not using (positive) upper limb support to maintain a challenging posture reinforcing of unsuccessful or successful attempts to increase weight shifting (Leech et al. 2022).

7 Compensatory Strategies

When people with ataxia move, they often demonstrate compensatory strategies of moving slowly or decomposition, moving one joint at a time (Bastian 1997). Compensations do not restore normal movement; however, compensations may improve function by reducing the interaction torques and producing less of a movement tremor and more accurate limb movements (Bastian et al. 1996). Compensatory strategies are often self-initiated by people with ataxia. For example, when people hold their elbows to their sides when lifting a cup to drink or walk more slowly because faster walking causes them to become unbalanced. In rehabilitation, these compensatory strategies may be employed with bracing the wrist in neutral flexion/extension or in supporting the ankle in a solid ankle-foot orthoses thereby reducing the movement at these joints reducing the number of joints being controlled in a motor task (Bastian 1997). Ankle foot orthoses may be more commonly used in people who are more impaired. People with moderate to more severe postural and gait ataxia will often require a wheeled walker over a cane due to difficulty with placement and imbalance. Because motor control requires an increased attentional demand for people with ataxia (Lang and Bastian 2002) simplifying complex tasks and reducing distractions is another important compensatory strategy. Recently, the long-practiced compensatory strategy of weighting a limb to help with limb ataxia has been shown to be ineffective in improving and may even worsen reaching (Zimmet et al. 2019; Manto et al. 1994). In the Bhanpuri example of hypometria above, limb weighting was hypothesized to correct for estimated and actual inertial mismatch, but Zimmet et al. demonstrated that this correction did not occur even with precise adjustments. Possibly the use of limb weighting has persisted because the weights may initially result in slowing down a movement which would improve motor performance.

8 Other Considerations

Response to rehabilitation interventions may depend on a host of other factors. Certain diagnoses may have greater potential to benefit from rehabilitation than others. Progressive cerebellar disease is presumably more amenable to rehabilitation strategies for improving performance earlier in the disease course and

compensatory strategies may be necessary later. Non-progressive cerebellar damage typically has a better prognosis; however, when the cerebellar nuclei are involved, recovery is poor (Bultmann et al. 2014; Schoch et al. 2006). In complex presentations, with other brain region involvement, response may be compromised or require additional supports.

While balance training is an essential component of rehabilitation to address gait ataxia. Balance exercises need to be safe but do-able and people with cerebellar disease need to have insight into their abilities and how to perform activities safely. As symptoms progress, often people report they are doing well because they haven't fallen but they do not realize they have widened their base of support to compensate for imbalance and are surprised when they cannot stand in tandem. To retrain balance, we would not want to continue to decompose a task, removing the balance demand. Additionally, balance exercise might be deferred until a person is strong enough and has enough endurance to sustain balance challenges so they can be successful and not fatigued from the activity alone.

Prescriptive dosing still needs to be optimized for treatment selection, frequency, duration, and level of exertion. Rehabilitation requires a knowledge of a person's abilities and limitations as well as barriers and facilitators to their compliance with exercise. A multidisciplinary approach with early referral on diagnosis and monitoring for disease progression is essential to optimize care. Updating and modifying a rehabilitation program needs to occur on at least an annual basis (Morton et al. 2010). Longer training periods may also be required due to impaired motor learning. People with mild symptoms may be appropriately managed with independent exercise intervention or with trained professionals such as exercise physiologists to maintain health and fitness. For people with progressive or more severe symptoms, rehabilitation professionals specializing in neurological disorders are indispensable to regain or maintain optimal physical function and independence and to prevent complications. Ideally, rehabilitation will be able to select and apply the optimal dose of task-specific training, using accessible learning mechanisms, at the appropriate time in a disease course to offer a targeted approach to treatment.

9 Future Directions

Studies using rehabilitation interventions for ataxia to date while few in number and sample size have also led to essential clinical recommendations and further questions to address. The Dynamic Gait Index and Action Research Arm Test have recently been shown to be reliable, validated measures for use with this population (Reoli et al. 2021a, b). Use of exergames is being used to enhance motivation and compliance with rehabilitation interventions for ataxia (Ayvat et al. 2022). Rehabilitation interventions may be augmented with emerging techniques in brain stimulation for greater benefit (Benussi et al. 2018). Virtual reality may be a means to be more precise in the intervention and dosing as well as provide reinforcement feedback to target preserved motor learning pathways (Therrien et al. 2021). Future

studies may address how reinforcement learning can be translated from the lab to clinical practice to improve retention of learning and generalization to new tasks. The large variability in gait parameters in cerebellar ataxia has been linked to falling (Schniepp et al. 2022); if balance exercises result in reduced gait variability the exercise may have a large impact to reduce falling and improve quality of life.

10 Summary

Rehabilitation for ataxia is both a standard of care and an evolving field of study for optimizing movement. The information presented here highlights the impairments caused by cerebellar damage and the resulting limb, balance, and gait deficits. Early management of symptoms is key to achieving improved outcomes therefore early referral to rehabilitation specialists is optimal. Management of deficits requires a team approach including re-evaluation at regular intervals to optimize rehabilitation intervention and provide appropriate care. As discussed, balance training is a key intervention to address gait dysfunction. Compensatory, fitness-oriented and modifiable interventions each have an important place in meeting the needs of people with ataxia.

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Part IV
Autosomal Recessive Cerebellar Ataxias

Recent Advances on Therapeutic Approaches for Friedreich's Ataxia: New Pharmacological Targets, Protein, and Gene Therapy

Deepika M. Chellapandi, Valentine Mosbach, Marie Paschaki, and Helene Puccio

Abstract Friedreich ataxia (FA) is an inherited autosomal recessive neurodegenerative disorder. The most common mutation in FA is caused by a (GAA)n triplet repeat expansion in the first intron of the frataxin gene. However, in 4% of patients, the disease is caused by a compound heterozygous GAA expansion with a loss of function mutation on the other allele. The genetic defect results in low levels of frataxin, which is an essential gene for mitochondrial function. FA is a multisystemic disorder primarily characterized by progressive sensory and spinocerebellar ataxia. In addition to neurological symptoms, many FA individuals also present a hypertrophic cardiomyopathy and diabetes. In this chapter, we discuss recent therapeutic approaches, including a proof-of-concept study for gene therapy, drug development targeting the affected downstream pathways, paving the way for the first disease-modifying therapeutic approaches.

Keywords Friedreich's ataxia · Mitochondria · Pathogenesis · Gene therapy · Pharmacology

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_23

1 Friedreich's Ataxia

1.1 Friedreich's Ataxia: A Multisystemic Disorder

Friedreich's ataxia (FA) is the most common inherited recessive ataxia with a prevalence around 1/50,000 people with a carrier rate being variable across populations (Delatycki and Corben 2012; Vankan 2013). This neurodegenerative disorder is characterized by a progressive spinocerebellar and sensory ataxia leading to gait disturbance, loss of coordination, and tremor of the upper limbs, dysarthria, scoliosis, muscular weakness, and spasms, extensor plantar responses, foot deformities (pes cavus), and areflexia (Delatycki and Corben 2012; Harding et al. 2020; Dürr et al. 1996). In most cases age of onset is before 25 years old (generally around 10–15) and patients are wheelchair-bound generally within 10–15 years of diagnosis (Puccio et al. 2014). In addition to neurological symptoms, a large proportion of FA individuals also present a cardiomyopathy and 10–30% present diabetes or glucose intolerance (Koeppen et al. 2015; Cnop et al. 2012). A myriad of other symptoms occur with disease progression, such as visual impairment or hearing loss, and are extremely variable from a patient to another (Fahey et al. 2008; Fortuna et al. 2009; Zeigelboim et al. 2018). Patients have a reduced lifespan, and complications due to the cardiomyopathy is the most common cause of death (Puccio et al. 2014; Tsou et al. 2011). While FA is a multisystemic disorder affecting non-neural tissues like endocrine pancreas or the heart with progressive left ventricular dysfunction, cardiomyocyte hypertrophy, and fibrosis, we can highlight three important neuronal sites: the dorsal root ganglia (DRG) with the progressive degeneration of the large sensory neurons and their axonal projection in the posterior columns, the spinocerebellar, and corticospinal tracts of the spinal cord, and the degeneration of the dentate nuclei of the cerebellum (Harding et al. 2020; Koeppen et al. 2017; Morral et al. 2010).

1.2 The Genetic Cause of Friedreich's Ataxia: From GAA Expansion to Gene Silencing

The genetic cause of Friedreich's ataxia was elucidated in 1996 with the discovery of a GAA trinucleotide repeats expansion within the 1st intron of the *FXN* gene coding the frataxin protein (Campuzano et al. 1996). The size of the GAA repeats is variable among the general population but never reaches more than 33 repeats. On the contrary in FA patients, repeat tracts between 44 and 1700 GAA are observed. Most FA patients (~96% of cases) are homozygous for the GAA expansion. The remaining 4% are compound heterozygous with one GAA-expanded allele and the other carrying a classical mutation (nonsense, missense, deletions, insertions) (Cossée et al. 1999; Galea et al. 2016). Like many others genetic diseases due to a microsatellite instability, an inverse correlation between the number of repeats, the

age of symptoms onset and severity is observed (Montermini et al. 1997a; Patel et al. 2016). In FA, the GAA expansion present both a meiotic and a mitotic instability (Delatycki et al. 1998; Monrós et al. 1997). During parental transmission, a bias is observed toward contractions of the repeat tract when alleles are passed from father to child whereas both expansions and contractions are observed for alleles passed from mother to child. Interestingly, although somatic instability is present in the central nervous system, it is much higher in the heart and pancreas (Montermini et al. 1997b; Long et al. 2017). In addition, the mitotic instability of GAA expansion increases with age (Long et al. 2017; De Biase et al. 2007; Bit-Avragim et al. 2001).

The presence of the GAA expansion induces a partial transcriptional silencing of the *FXN* gene, leading to a reduced level of the FXN protein around 5–30% in FA patients (Bidichandani et al. 1998; Campuzano et al. 1997). A negative correlation between GAA repeats length and transcript levels is observed (Chutake et al. 2014a). Although the precise(s) molecular mechanism(s) by which GAA-expanded mediate gene silencing is not fully understood, evidences of repressive chromatin formation resulting in deficiency of transcriptional initiation and elongation were found (Chutake et al. 2014b; Kumari et al. 2011; Li et al. 2015). Reduced levels of active chromatin marks such as histone hypoacetylation (H3K9, H3K4, H4K5) were observed, while heterochromatic marks like histone di- or tri-methylation were increased and spread upstream the GAA repeat in the 1st intron toward the promoter (Chutake et al. 2014a; Kumari et al. 2011; Al-Mahdawi et al. 2008; Soragni et al. 2014). DNA methylation was also detected upstream the repeat tract (Al-Mahdawi et al. 2008; Castaldo et al. 2008; Evans-Galea et al. 2012). Recently, deep sequencing analysis allowed to distinguish phenotypic groups of FA patients based on the proportion of *FXN* gene lacking silencing signal in somatic cells (Rodden et al. 2021). In addition, expanded-GAA have been also shown to form in vitro non canonical structures such as DNA triplexes and to stimulates the formation of R-loop structure (DNA-RNA hybrid) which could interfere with transcription, reduce accessibility of transcriptional regulatory factor and trigger repressive chromatin formation, but there is no evidence yet that the same type of structures could be formed in vivo *(*Bidichandani et al. 1998*;* Li et al. 2019*;* Groh et al. 2014*;* Mikaeili et al. 2018*)*.

2 Frataxin Plays a Major Role in Fe-S Clusters Biogenesis

2.1 The Frataxin Protein

Frataxin is a highly conserved protein, from bacteria to human. In eukaryotes, it is nuclear-encoded gene which codes for a protein precursor of 210 amino acid, that is imported into the mitochondria and matured by a two steps maturation producing the full mature and functional form (FXN81-210) (Schmucker et al. 2008; Condò et al. 2007). Although, frataxin is known to be an iron-binding protein and has been

suggested to be involved in a variety of pathways link to iron metabolism, transport, storage, or heme synthesis, its precise role remains unclear (Foury et al. 2007; Yoon and Cowan 2003, 2004). The only function widely accepted of frataxin is its essential role as a regulator of Fe-S clusters biogenesis.

2.2 *Fe-S Clusters Biogenesis: A Conserved Mechanism Driven by Frataxin?*

Fe-S clusters are essential inorganic cofactors required for a variety of proteins involved in key cellular processes including central metabolism and respiration, DNA replication and repair, ribosome biogenesis or tRNAs modification (Rouault 2015, 2019; Lill 2009) (Fig. 1). Even if ferrous iron and sulfide can spontaneously assemble a simple Fe-S cluster under reducing conditions, free iron and sulfur are toxic for the cell and Fe-S clusters are sensitive to oxygen. Conserved protein machineries have been found in all kingdom of life to facilitate clusters assembly and their insertion into apo-protein. In eukaryotes, the key step initiating Fe-S clusters biogenesis is the de novo assembly of the iron and sulfur in mitochondria by the multiprotein complex ISC, mainly composed of the NFS1 cysteine desulfurase which provide the inorganic sulfur, the scaffold protein ISCU on which the cluster is assembled, the ferredoxin protein (FDX2-FDXR couple in mammals) which provide electrons and the frataxin protein thought to regulate complex activity (Beilschmidt and Puccio 2014; Braymer et al. 2021). How the iron is provided to this multiprotein complex is still under investigation.

Most of our knowledge on the biochemistry process of the ISC complex comes from in vitro analysis, crystal structure resolution, and reconstitution kinetics using purified proteins. It was rapidly determined that the cysteine desulfurase NFS1 convert a L-cysteine to L-alanine by generating a persulfide intermediate on a conserved cysteine residue of a mobile S-transfer loop which is transferred to a cysteine residue on the scaffold protein ISCU (Beilschmidt and Puccio 2014; Braymer et al. 2021). This catalytic process has been shown to be dependent on a pyridoxal-phosphate mechanism that requires direct interaction of NFS1 with both a LYMR superfamily protein ISD11 and an acyl carrier protein ACP, stabilizing the cysteine desulfurase and forming the ternary complex NFS1/ISD11-ACP/ISCU (Wiedemann et al. 2006; Adam et al. 2006). The frataxin protein is known to directly interact with this ternary complex, forming a quaternary complex, and was proposed to play a regulatory role either by stimulating binding of cysteine substrate to NFS1 or by stimulating sulfide transfer from the cysteine desulfurase to the scaffold protein ISCU (Schmucker et al. 2011; Fox et al. 2015; Parent et al. 2015). In addition, this complex was shown to form a symmetric hetero-octamer composed of a NFS1/ISD11/ACP homohexamer core with two ISCU proteins bound to each end and two FXN proteins that occupied a cavity at the interface between each NFS1 and ISCU

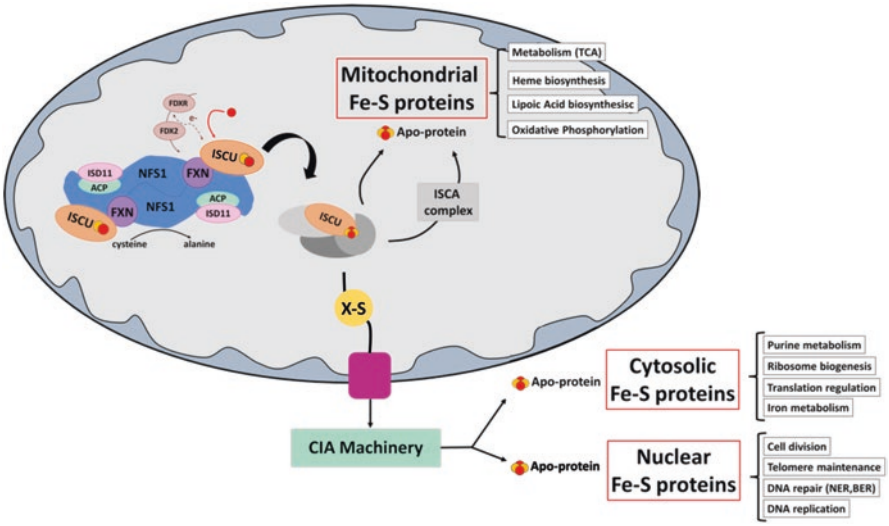

Fig. 1 Fe-S clusters biogenesis in mammalian cell. Assembly of iron and sulfur is performed in mitochondria by a hetero-octamer machinery (ISC) composed of: two cysteine desulfurize NFS1 (sulfur donor), two LYMR protein-acyl carrier protein ISD11-ACP (stabilize NFS1), two scaffold protein ISCU (where the cluster is assembled) and two frataxin protein FXN (allosteric regulator). The iron donor is unknown. Frataxin protein will accelerate each step of the reaction from iron entry to persulfate formation by NFS1 and its passing to a conserved cysteine residue on ISCU via a mobile S-transfer loop. After generation of a bridging [2Fe-2S] clusters on ISCU, it is released to glutaredoxin and chaperone proteins to be either directly deliver or give to the ISCA complex to form [4Fe-4S] clusters for mitochondrial apo-proteins. Alternatively, a sulfur-compound of unknown nature will be exported outside mitochondria, the cytosolic Fe-S assembly machinery (CIA) for the delivery into cytosolic and nuclear apo-proteins. A non-exhaustive list of major cellular pathways that contain Fe-S clusters proteins are represented for each cellular compartment

(Boniecki et al. 2017; Cory et al. 2017) (Fig. 1). Recent findings tend to precise FXN function. Radiolabeling assay and stopped-flow kinetics showed that FXN is functionally linked to the mobile S-transfer loop cysteine of NFS1 by specifically accelerating each step from substrate binding to persulfide formation through pyridoxal-phosphate and sulfur delivery agent to the scaffold protein (Patra and Barondeau 2019). In support of this data, the recent structure by cryo-electron microscopy of human ISC complex showed that one FXN protein contacts one of the two NFS1 protomer at the catalytic S-loop (Fox et al. 2019). In summary, recent developments allowed to improve our knowledge on the mechanistic of the ISC complex, underlying the importance of iron binding on ISCU as an early step of the process and the major role of frataxin protein to enhanced the sulfur transfer reaction from NFS1 to ISCU (Maio et al. 2020; Campbell et al. 2021).

2.3 ISC Deficiency on Fe-S Clusters Delivery

After assembly, the cluster is released form the scaffold protein to a glutaredoxin and chaperone proteins that will mediate the delivery either directly to [2Fe-2S] mitochondrial targets or to a second complex, the ISCA complex, which will mediate formation and transfer of [4Fe-4S] clusters into mitochondrial proteins targets. Alternatively, a sulfur-compound of unknown nature will be exported outside the mitochondria through a specific transporter to the cytosolic Fe-S assembly machinery for the delivery into cytosolic and nuclear apo-proteins (Braymer et al. 2021; Saha et al. 2018) (Fig. 1).

Therefore, the ISC complex is at the center of Fe-S clusters biogenesis which not only supplies clusters to the mitochondria but also provides an essential compound for clusters assembly and delivery to cytosolic and nuclear targets. The frataxin protein has been shown to be essential, its absence leading to embryonic lethality in mice and plants, to larval stage arrest in *Caenorhabditis elegans* and reduced larval viability in the *Drosophila*, underlining the importance of its partial silencing in FA (Cossée et al. 2000; Ventura et al. 2006; Anderson et al. 2005). Low levels of frataxin protein will lead to a strong decrease of Fe-S clusters biogenesis that will impede not only mitochondrial but also cytosolic and nuclear proteins involved in specific pathways and constituting the molecular basis of the pathology.

3 Cellular and Molecular Pathogenesis

3.1 Hallmarks of FA Pathophysiology: A Vicious Cycle Empowered by Fe-S Deficit

Prior to the discovery of FXN function, it was already identified that the main hallmarks of FA pathophysiology were mitochondrial dysfunction, dysregulation of iron homeostasis, impaired antioxidant defense pathways, oxidative stress, and inflammation (Puccio et al. 2014; Clark et al. 2018; Delatycki and Bidichandani 2019). These phenotypes are strongly linked together since it is well known that impairment of oxidative respiration or iron dysregulation (through Fenton reaction) can result in increased levels of reactive oxygen species (ROS). The characterization of frataxin function in Fe-S biogenesis further highlights the role of Fe-S proteins deficit in the overall pathophysiology, adding to the complexity of deciphering the primary cause(s) of the pathology. Deficit in Fe-S proteins involved in oxidative respiratory chain, Krebs cycle, and iron homeostasis has been observed across FA tissues, cells lines, and models (Puccio et al. 2014; Clark et al. 2018; Delatycki and Bidichandani 2019; Martelli and Puccio 2014). Therefore, the pathophysiology of FA is often described as a vicious circle starting by the lack of frataxin leading to Fe-S clusters loss, iron overload, and oxygen radical production which are mutually exacerbating each other (Fig. 2a). However, many of the therapeutical strategies

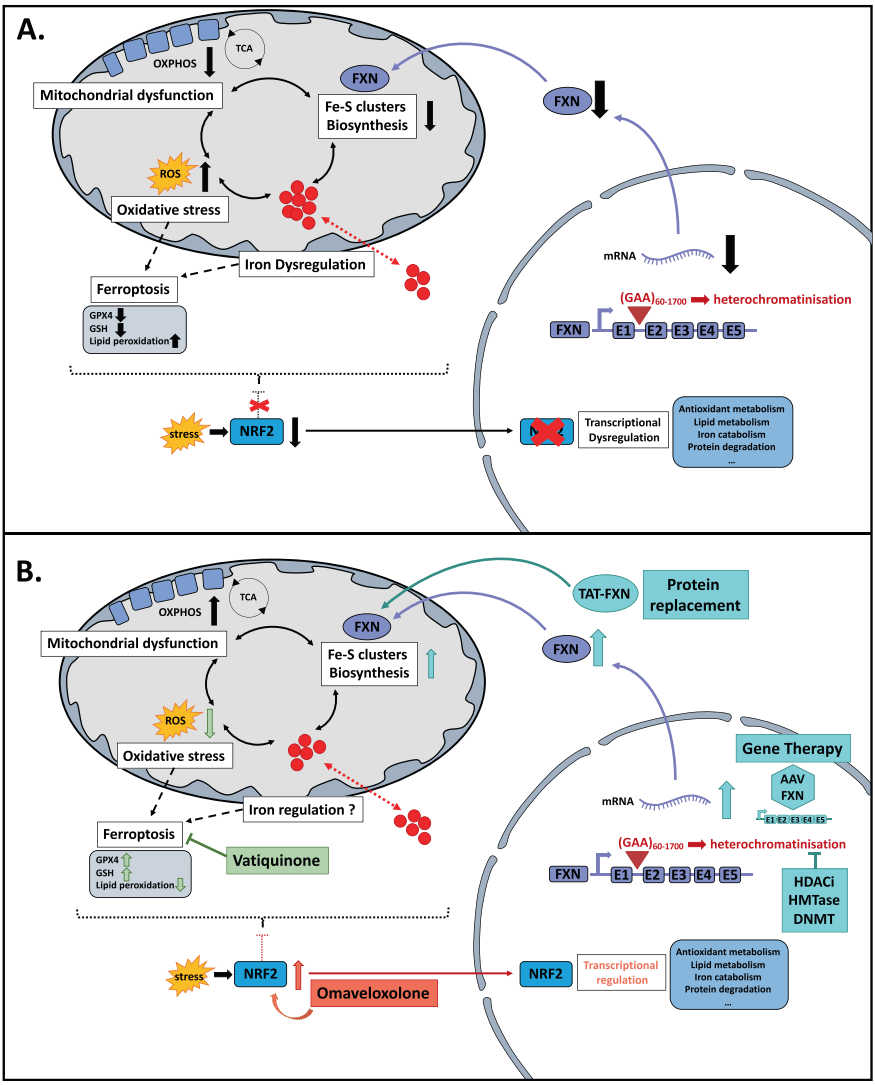

Fig. 2 Pathophysiological pathways and therapeutics targets in Friedreich ataxia. (**a**) Main physiological pathways affected in FA. GAA expansion in the 1st intron of *FXN* gene leads to decrease production of FXN protein through heterochromatinization of the locus. Deficit in FXN leads to global mitochondrial dysfunction by starting a vicious circle of Fe-S clusters loss, iron overload, and oxygen radical production which mutually exacerbating each other. The iron-dependent ROS overload generating lipid peroxidation in addition of impaired glutathione metabolism will ultimately lead to cell death through ferroptosis. The transcription factor NRF2, known as a master regulator of stress, has been shown to be downregulated and to translocate to the nuclei under stress conditions in FA models, increasing the previously mentioned phenotypes. (**b**) Different therapeutic strategies for FA. (1) Targeting downstream events: Omaveloxolone will increase *NRF2* expression and restore its translocation in the nuclei; Vatiquinone will act as an antioxidant and inhibits ferroptosis. (2) Increasing frataxin level: Epigenetic-based therapeutics (HDACi, HMTase, DNMT) to abolish the heterochromatinization; Gene therapy using AAV vector-hFXN cDNA; Protein replacement therapy to deliver exogenous TAT-FXN directly in the mitochondria

targeting specifically the oxidative stress by antioxidant or the iron dysregulation by chelator have not been conclusive (see Part IV), suggesting that the pathophysiology is probably more complex. The molecular pathways characterized and related to oxidative stress, iron, and mitochondria metabolism in FA have been extensively reviewed before (Puccio et al. 2014; Clark et al. 2018; Delatycki and Bidichandani 2019; Martelli and Puccio 2014). However, there are probably a lot of unidentified pathways that may play a role that remain to be elucidated. Whether dysregulation of DNA metabolism or translation are also involved in the pathophysiology of FA remains to be further validated considering the variety of Fe-S proteins involved in these pathways in the nucleus or the involvement of ROS production and genotoxic stress produced by the unhealthy mitochondria leads to mitochondrial DNA damage that may altered genomic DNA transactions such as transcription or replication (Culley et al. 2021; Anjomani Virmouni et al. 2015; Moreno-Lorite et al. 2021). Here, we will only discuss pathways and mechanisms recently found related to FA pathology that could present a relevance for therapeutic approaches.

3.2 Ferroptosis: A Major Cell Death Mechanism in FA?

Unbalanced of iron metabolism is one of the key features of FA whose mechanistic contribution to the pathophysiology is complex to decipher, but emerging data point out its driving role in a newly identified programmed cell death pathway, ferroptosis, that may be a key mechanism in FA. This pathway, first described in 2012, is trigger by iron-dependent ROS overload generating lipid peroxidation and impaired glutathione metabolism which leads to morphologic and biochemical changes distinct from apoptosis and other necrotic pathways (Dixon et al. 2012). One of the major actors playing a protective effect against ferroptosis is the glutathione Peroxidase 4 (GPX4), an antioxidant that prevents membrane lipid peroxidation by using reduced glutathione (GSH) (Seibt et al. 2019). Interestingly, all ferroptosis inducers tested so far acts directly or indirectly on GPX4 expression or activity supporting its relevance (La Rosa et al. 2020a). All the main features leading to ferroptosis are found in FA from iron-induced oxidative stress to lipid peroxidation (Turchi et al. 2020a) (Fig. 2a). A depletion in GSH has also been observed in fibroblast from patients as well as in several models, and a decrease in GPX4 expression was found in embryonic fibroblasts from the KIKO mice model (Table 1) (Piemonte et al. 2001; Petrillo et al. 2019; Turchi et al. 2020b; Ocana-Santero et al. 2021). Moreover, a recent work showed that fibroblasts of FA patients as well as several murine-based cell models are hypersensitive to erastin-treatment, a ferroptosis inducer which inhibits GSH synthesis (Cotticelli et al. 2019). This study was also able to demonstrate a protecting effect of ferroptosis inhibitors on human and mouse FA cells treated with ferric ammonium citrate and an inhibitor of GSH synthesis, whereas apoptotic inhibitors failed (Cotticelli et al. 2019). In addition, Fe-S clusters biogenesis machinery deficiency was shown to increased ferroptosis sensitivity through activation of iron-starvation response via the Iron Regulatory Protein 1, known to be

Table 1 Friedreich's ataxia mouse models mentioned in the present review

Potential use	Mouse model	Description	Reference
Gene therapy; frataxin replacement therapy; effects of frataxin loss in a tissue dependent manner	***Conditional mouse model***		
	MCK-cKO	Conditional knockout in heart and skeletal muscle using a cre-recombinase under the muscle creatine kinase promoter. Reduced lifespan (76 ± 10 days). Hypertrophic cardiomyopathy. No skeletal muscle phenotype. Early Fe-S cluster deficit and late mitochondrial iron accumulation.	Puccio et al. (2001)
	NSE-cKO	Conditional knockout in nervous system, heart and liver using a cre-recombinase under the neuron-specific enolase promoter. Reduced lifespan (29 ± 9 days). Severe neuronal and cardiac phenotype	Puccio et al. (2001)
	Pvalb-cKO	Conditional knockout in parvalbumin positive cells (Proprioceptors, Purkinje Neurons, Cortical Interneurons) using a cre-recombinase under the parvalbumin promoter. Develops mixed sensory and spinocerebellar ataxia, loss of Fe-S cluster. Reduced lifespan 22 weeks.	Piguet et al. (2018)
	Ins2-cKO	Conditional knockout in pancreatic β-cells using a cre-recombinase under the Insulin promoter. Develops diabetes mellitus	Ristow et al. (2003)
	ALB-cKO	Conditional knockout in hepatocyte using a cre-recombinase under the Albumin promoter. Tumor formation or liver regeneration	Thierbach et al. (2005), Martelli et al. (2012)
	Mouse models with point mutation		
	G127V	Harboring a point mutation replacing evolutionarily conserved glycine 127 to valine in mouse FXN. Reduced levels of frataxin protein. Embryonic fibroblasts exhibit significantly reduced proliferation and increased cell senescence. Increased frequency of mitochondrial DNA lesions and fragmentation	Fil et al. (2020)
Functional studies based on FXN mRNA or protein level rescue, and for epigenetic levels	***Mouse models with GAA expansions***		
	KIKI	Double knock-in with 230 GAA repeats. No overt phenotype. Transcriptional deregulation involving PPARg pathway. Markers of heterochromatin on the GAA tract	Miranda et al. (2002)
	KIKO	Simple knock-in crossed with knockout mouse. 26–32% residual frataxin expression. No overt phenotype. Transcriptional deregulation involving PPARγ pathway	Miranda et al. (2002)
	YG8R, YG22R, YG8sR, YG8 800	YAC containing the full human *FXN* locus with a GAA expansion and deleted for endogenous murine frataxin. Progressive ataxia with affected DRG. No cardiopathy but mitochondrial iron accumulation and lipid peroxidation. Markers of heterochromatin on the GAA tract. Tissue-dependent GAA instability	Al-Mahdawi et al. (2006)

For complete information on all mouse models available in the field, see Ocana-Santero et al. (2021) and http://curefa.org/pdf/research/MouseModels-inFA.pdf

an Fe-S protein, and more surprisingly via Iron Regulatory Protein 2 (Terzi et al. 2021). However, this was performed in cancer cell lines, and this should be explored in FA models. Even if investigations on the relationship between ferroptosis and FA pathophysiology are still emerging comparing to other neurodegenerative diseases for which it is more well established, such as Huntington or Alzheimer disease, it appears like a promising major pathway for both understanding the pathology and for new targets for drug development (Cheng et al. 2021). Notably, a potential interesting target is the master regulator nuclear factor erythroid 2-Related Factor 2 (NRF2) involved in multiple cellular processes including ferroptosis and more generally antioxidant defense (see Part IV).

3.3 NRF2: The Master Regulator of Stress Is Affected in FA

Nuclear factor erythroid 2-Related Factor 2, a member of the cap'n'collar subfamily of basic region leucine zipper transcription factor family, regulates the expression of at least 250 genes through a specific cis-acting enhancer sequence found in the promoter region and called the Antioxidant Response Elements (Nguyen et al. 2009; Niture et al. 2014). Under basal condition, NRF2 is sequestered in the cytoplasm by KEAP1, which controls the ubiquitin-dependent degradation of NRF2, while as a response to stress, NRF2 translocates to the nucleus where it will interact with small MAF proteins and binds its targets (Bryan et al. 2013). Genes controlled by NRF2 encode for enzymes found in many cellular processes such as antioxidant metabolism, lipid metabolism, iron catabolism, protein degradation, or regulators of inflammation (Nguyen et al. 2009; La Rosa et al. 2020b). Therefore, NRF2 can be considered as a master regulator capable of coordinating various responses on different types of cellular stress. The involvement of NRF2 antioxidant signaling pathway in FA was first reported more than 10 years ago in fibroblasts derived from FA patients that failed to translocate NRF2 in the nucleus in response to oxidative stress and that presented a decrease expression of some NRF2 targets like SOD2 or NQO1 (Paupe et al. 2009) (Fig. 2a). Since then, evidences of decrease expression and protein levels of NRF2, failure of its translocation in nuclei under stress condition and decrease expression of many of its targets, have been found in several FA models in vitro such as neurons silenced for *FXN* as well as in vivo, notably in YG8R and *Mck* cKO mouse models (respectively FXN YAC transgenic model and conditional cardiac model, see Table 1 and Box 1) (D'Oria et al. 2013; Shan et al. 2013; Petrillo et al. 2017; Anzovino et al. 2017). Recently, studies came through showing NRF2 as a potential interesting therapeutic target for FA. Increasing expression and activity of *NRF2* in neuronal stem cells derived from KIKO mouse model (see Table 1 and Box 1) restored neural stem cell differentiation program and associated phenotypes (La Rosa et al. 2019). The use of several drugs targeting the NRF2 pathway in fibroblasts from FA patient allowed to evaluate their efficacity for potential treatment, and notably highlights the effect of the new drug Omaveloxolone (see Part IV) (Petrillo et al. 2019). Moreover, NRF2 is known to regulate multiples genes

Box 1 Potential Uses of Each Mouse Model

The complete knockout of *Fxn* in mice is embryonic lethal (Cossée et al. 2000). The first viable mouse models of FA were generated using conditional approaches using the Cre-LoxP recombination system to perform deletion of *Fxn* exon 4 from a conditional floxed allele (Fxn^{L3}) using tissue-specific, Cre mouse lines. Cardiac-specific (*MCK-Cre*) and neuronal (*NSE-Cre*, *Pvalb-cKO*) models of FA were obtained (Table 1). Conditional knockout mice have severe cardiac or neuronal phenotypes. This is because the endogenous gene is ablated to recapitulate an FA-like phenotype, although to a more severe extent, since there is a complete absence of frataxin in the tissues of interest. These animal models are perhaps the best existing models for studies involving gene therapy and protein replacement strategies and to understand tissue-specific downstream events of frataxin deficiency.

Although conditional knockout mice are powerful tools to reproduce the disease pathophysiology in heart and the nervous system, and to test some therapeutic approaches, they do not perfectly mimic the human disease. In patients, the presence of a GAA expansion on at least one *FXN* allele leads to low levels of structurally normal frataxin (Campuzano et al. 1996). The progressive disease thus results from the presence of a residual amount of frataxin throughout life, rather than a sudden and complete absence of frataxin. Furthermore, the genetics of GAA expansions might contribute to disease development, possibly by having a role in tissue specificity owing to the intrinsic somatic instability of long GAA tracts. In addition, GAA-based mouse models are needed to unravel the molecular and cellular mechanisms associated with GAA-mediated silencing of the *FXN* gene in vivo, as well as for the therapeutic evaluation of drug candidates that might target this process. GAA-based mouse models were obtained using either a knock-in approach based on homologous recombination or a human genomic yeast artificial chromosome (YAC) transgenic approach. Two lines of human genomic YAC FA transgenic mice (YG22 and YG8) that contain unstable GAA-repeat expansion YG22 (GAA_{190} and GAA_{190+90}, respectively) within the appropriate genomic context rescue the embryonic lethality of the knockout by expressing only human frataxin (Al-Mahdawi et al. 2006). The YG8R mice (Table 1) exhibit a reduced expression of human frataxin protein and display mildly impaired motor coordination ability with evidence of large neurodegeneration vacuoles in the DRG and decreased aconitase activity in the cerebellum. No severe heart dysfunction was observed in the YG8R mice. However, decreased aconitase activity, mitochondrial iron accumulation, and signs of lipid peroxidation suggestive of ROS formation were found in heart tissue (Al-Mahdawi et al. 2006). Generating adequate GAA-based models that lead to the development of a FA phenotype in a mouse is not an easy task. The main difficulties lie in the length of the GAA expansion needed to induce pathogenesis, the locus of genomic integration, as well as the intrinsic GAA instability that can

result in contraction or elongation of the expansion. Both KIKI and YG8R mice are powerful tools to study the mechanisms involved in GAA-mediated silencing and GAA somatic instability (YG8R). To obtain new mouse models with a more severe phenotype, current efforts are being made to increase the size of the GAA expansion within the human *FXN* transgene

involved in ferroptosis, like GPX4 or genes required for synthesis and recycling of GSH. Considering its downregulation in many FA models and the recent insights presenting ferroptosis as a key pathway of cellular death in the pathology, NRF2 may play a protective effect increasing its relevance as a therapeutic target. Supporting that idea, a recent study has shown that NRF2 inducers helped to overcome ferroptosis hallmarks found in FXN-silenced mouse myoblasts but also in fibroblasts and blood of FA patient (La Rosa et al. 2021).

4 Treatment Strategies Targeting Downstream Events

Etiology of FA includes an imbalance in intracellular iron homeostasis, mitochondrial deficiency, and sensitivity to oxidative stress, therefore targeting and ablating the secondary effects of loss of frataxin is one therapeutic approach explored in FA. The initial therapeutical approaches developed were focused on iron chelators or antioxidant drugs. Numerous studies have been conducted in vivo and in vitro across different FA models to test these diverse molecules, among them we can name idebenone, a short-chain Coenzyme Q_{10} acting on OXPHOS and protecting from lipid peroxidation, or the iron chelator deferiprone that quickly redistributes iron in the CNS (Puccio et al. 2014; Delatycki and Bidichandani 2019; Kearney et al. 2016). However, neither these two molecules nor related medicines have been proven effective in clinical studies. For a full review of all therapeutic approaches present in FA, please refer to the FARA pipeline (https://curefa.org/pipeline) and to the multiple reviews that have been recently written (Beaudin et al. 2022; Yang et al. 2022; Zesiewicz et al. 2020). We decided to focus in this review on some of the active molecules that are most promising and are currently in Phase III of clinical studies that targets the affected downstream molecular pathways discussed above that were recently discovered and are more relevant to the pathophysiology of the disease.

4.1 Omaveloxolone: Targeting the NRF2 Pathway

Omaveloxolone is a synthetic compound that is a second-generation member of oleanane triterpenoid (Reisman et al. 2014a). It has been shown to increase gene expression of *NRF2* and decrease expression of Nuclear Factor kappa-light-

chain-enhancer of activated B cells (*NF-κB*) (Reisman et al. 2014b). As previously discussed, *NRF2* is a key regulator of stress found downregulated in FA. Thus, targeting NRF2 pathway in FA could help with restoring the cellular phenotypes (Lynch and Johnson 2021). Several studies showed that using the molecule Omaveloxolone in FA cells derived from patients or mice models could improve mitochondrial functions, restore redox balance, and reduce inflammation, by increasing *NRF2* expression and decreasing *NF-κB* expression (Petrillo et al. 2019; Abeti et al. 2018) (Fig. 2b). Omaveloxolone was until recently in clinical trials and constitute one of the most promising pharmaceutical drug molecules. An international double-blinded, randomized, placebo-controlled Phase II clinical trial was conducted for 48 weeks on a total of 155 patients with a dosage of 150 mg/day and placebo in a 1:1 ratio (Reata Pharmaceuticals, Inc. 2021). Patients for the trial were selected based on the modified Friedreich's Ataxia Rating Scale (mFARS), only patients with a score of 20–80 were approved for the treatment. After 48 weeks of treatment, they observed an increase in mFARS score for patients treated with Omaveloxolone (Lynch et al. 2019; Rodden and Lynch 2021). On February 28th 2023, Reata announced that the U.S. Food and Drug Administration has approved SKYCLARYS™ (omaveloxolone) for the treatment of Friedreich's ataxia in adults and adolescents aged 16 years and older. This is the first therapy specifically indicated for the treatment of FA. In the U.S. SKYCLARYS has received Orphan Drug, Fast Track, and Rare Pediatric Disease Designations from the FDA. Additionally, the company's Marketing Authorization Application for omaveloxolone is under review in Europe by the European Medicines Agency (EMA). The European Commission has granted Orphan Drug designation in Europe to omaveloxolone for the treatment of Friedreich's ataxia.

4.2 Vatiquinone (EPI-743): A Cytoprotective Effect Against Ferroptosis

Vatiquinone, granted Orphan Drug for FA, is a vitamin E derivative metabolite of α-tocotrienol which presents antioxidant properties and a strong ferroptosis inhibitory activity (National Human Genome Research Institute (NHGRI) 2021) (Fig. 2b). This molecule inhibits the enzyme 15-lipoxygenase, a key regulator of oxidative stress and inflammation response pathways, but also targets *NQO1* resulting in an increased of glutathione biosynthesis (Feng et al. 2020). Using Vatiquinone to target ferroptosis is an interesting therapeutic strategy for mitochondrial disorders demonstrated in in vitro studies performed in cells derived from patients with mitochondrial disease-associated epilepsy that showed reduced levels of ferroptosis and a cytoprotective effect after treatment (Kahn-Kirby et al. 2019; PTC Therapeutics 2022). Vatiquinone has been evaluated in several clinical studies for FA. A 24-month, double-blinded randomized study demonstrates that it is a safe and well-tolerated drug and also shows a statistically significant improvement in neurological function

(Zesiewicz et al. 2018; PTC Therapeutics 2022). Phase II trial with 60 FA participants randomly administered with Vatiquinone demonstrated a statistically significant effect on disease severity at 18 months relative to age and stage-matched natural history controls as assessed by the validated FARS score and a favorable safety profile (Zesiewicz et al. 2018). Recently, PTC therapeutics has announced the initiation of Global phase III trial called MOVE-FA, an 18-month study to evaluate the effects of the molecule in children and young adults with FA having the primary end-point with assessment scores based on mFARS. Even though studies show improvements in cellular and mitochondrial function, but also in neurological state, there has been no significant improvements observed in cardiomyocytes nor studies showing an effect of the molecule in heart. Future of this molecule as a potential cytoprotective compound for FA will depend on the observations of Phase III trial. Details on clinical studies follow-up are available at https://ir.ptcbio.com/news-releases/news-release-details/ptc-therapeutics-announces-initiation-global-phase-3-clinical.

5 Therapeutic Approaches Aimed at Increasing Frataxin Levels

Uncovering the genetic origin and the pathophysiological processes involved in FA onset and progression allowed to propose novel therapeutic approaches. Since FA is characterized by low FXN levels in all tissues, an obvious strategy to attenuate FA related symptoms was to restore FXN levels. In order to achieve this aim, several studies reported either epigenetic-based strategies, gene therapy, and protein replacement approaches. We discuss a non-extensive overview of the current status of those approaches below.

5.1 Current Epigenetic-Based FA Therapeutic Strategies

Epigenetics targets to restore *FXN* transcription have been extensively investigated since 2007, with a significant active investigation since 2014 (Soragni et al. 2014; Gottesfeld 2007). Human patient derived cell models as well as GAA-based mouse models have been used in the past 15 years to discover and test the effects of epigenetic drug treatments. However, this approach is still under investigation and has not led to an efficient drug discovery. Indeed, although large progress has been made in understanding the epigenetics modification as well as finding novel target to modulate gene expression, the complexity of the *FXN* gene regulation makes its upregulation delicate to control. CRISPR/Cas9 technology allowed to remove the (GAA)n region in FA patient hemopoietic cells; however, the requested tissue-specific effects have not yet been reached (Rocca et al. 2020). An extended overview of available

active molecules and their limitations is reviewed in Yang et al. (2022). In terms of drug discovery strategy, scientific studies have been focusing on targeting chromatin-remodeling enzymes, such as histone deacetylase (HDAC) and DNA methyltransferase (DNMT) to increase frataxin transcriptional levels. Several small molecules have been used in vivo and in vitro as a proof of concept of epigenetic-based therapy (Soragni and Gottesfeld 2016).

As heterochromatin is a key factor in pathogenic *FXN* gene silencing, chromatin acetylation by HDAC inhibition, using chemical compounds (HDACi) such as 2-aminobenzamide, has been described to efficiently increase *FXN* locus transcription in patient's cells (PBMC and lymphocytes) and in various cell and animal models. In particular, compound 109 has been tested in preclinical studies. Ex vivo studies demonstrate that HDACi increases *FXN* mRNA levels in patient lymphoblastoid cell lines and peripheral blood mononuclear cells (PBMCs), but these effects remain transitory and imply repetitive dug treatments that eventually lead to cell toxicity (Herman et al. 2006; Rai et al. 2010; Plasterer et al. 2013). Compound 109 and derivatives show suboptimal brain penetrability and more importantly give rise to toxic metabolites. Both biodistribution and toxicity need to be improved in order to consider an effective therapeutical approach. Therefore, new molecules are currently being developed and are under investigation. Active molecules such as hydroxamic acids, toluene sulfonic acid, and suberoylanilide hydroxamic acid have not been conclusive causing toxic side effects (Herman et al. 2006). Since more than 18 HDAC enzymes have been identified in the human genome, most active molecules display off target effects known since 2016 (Soragni and Gottesfeld 2016). However, in 2021, a review of molecules under clinical trials clearly indicates that HDACi remains still a therapeutic route to explore (Bondarev et al. 2021).

Since patient lymphoblastoid cells and fibroblasts display H3K27me3 increased levels, the effects of potential Histone Methyltransferase (HMTase) inhibitors have been under investigation (Ziemka-Nalecz et al. 2018; Wang and Liu 2019). Among chemical components, GSK126 has been described for being an effective and specific inhibitor (McCabe et al. 2012). It specifically inhibits EZH2, which catalyzes H3 methylation process and indirectly leads to *FXN* mRNA increase. Since H3K9me2 levels are increased in FA patients, then one can hypothesize that HMTase inhibitors would lead to an effective treatment. BIX-01294 compound has been shown to be effective in FA lymphoblastoid cells, by depleting H3K9me2 signals at the expanded GAA repeats. However, it fails to increase *FXN* transcriptional levels, suggesting a redundancy in H3K9me2/me3 action (Punga and Bühler 2010).

Friedreich Ataxia Research Alliance (FARA) pipeline (https://curefa.org/pipeline) resumes actual research on increasing *FXN* gene expression as mostly being on discovery or pre-clinical development. Oligonucleotides are currently studied in order to target (GAA)n repeats so that *FXN* expression can be restored in affected tissues. Synthetic transcription elongation factors are also under clinical trials (phase 1) since February 2022. These data indicate that epigenetic regulation remains a crucial point for developing FA therapy.

5.2 *Gene Therapy*

The first frataxin gene transfer study was conducted on a localized FA conditional mouse model, in which frataxin was deleted in inferior olivary nucleus neurons by injecting herpes simplex virus (HSV-1) that expresses the Cre-recombinase (Lim et al. 2007). After 4 weeks of frataxin deletion, the mice started showing behavioral defects in motor coordination. When these affected mice were injected with HSV-1 amplicon expressing human frataxin complementary DNA (cDNA), a reversal in the phenotype was observed with a physiologically relevant level of endogenous *FXN* expression (Lim et al. 2007).

Furthermore, a proof-of-concept study for cardiac gene therapy showed the capacity of intravenous injection of an AAV vector expressing human frataxin under a ubiquitous promoter (AAVrh10.CAG-hFXN-HA) to not only prevent the onset of cardiac disease but also to present a complete reversal of cardiac phenotype after post-symptomatic injection in the *MCK* cKO mouse model (Perdomini et al. 2014). At the molecular level, a full recovery of Fe-S cluster biosynthesis, normalization of the pathology-induced gene program in treated *MCK* cKO mice, and decreased interstitial cardiac fibrosis were immediately seen after treatment (Perdomini et al. 2014). This original study was confirmed by other studies in cardiac mouse models with different serotypes and promoters demonstrating that exogenous frataxin expression can reverse the phenotypes (Gérard et al. 2014; Salami et al. 2020). In particular, one study proposed to investigate the relevancy to the time when the gene therapy would be most effective in a stress-induced moderate cardiac-specific FA model (Salami et al. 2020). At rest, no clinical phenotypes are visible in this model but a severe cardiac dysfunction occurs when exposed to chemical and exercise-induced stress. The authors demonstrated that the cardiac dysfunction in response to synthetic and exercise-induced stress could be treated with an AAV-based gene therapy using the AAVrh.10 serotype (Salami et al. 2020).

These proof-of-concept studies were achieved with high-dose AAV vectors that are not replicable in clinical conditions for adult patients with the existing delivery methods. Interestingly, a dose-response study using the AAVrh10.CAG-hFXN-HA vector in the *MCK* cKO mice reveals that 50% transduction of cardiomyocytes was sufficient for full recovery of cardiac function and molecular characteristics of the disease (Belbellaa et al. 2019). The study was performed at two different time points to compare early and late stages of cardiac dysfunction. Early stage of mild left ventricle systolic dysfunction is observed in 5 weeks old mice and at 7 weeks left ventricle is substantially dilated and hypertrophied. Meaningful therapeutic effects were observed with 50% transduction of cardiomyocytes which is proved to be achieved by a cell autonomous behavior. Additionally, the concentration of hFXN therapeutic threshold was within the physiological range, implying that just only a small increase in FXN is required (Belbellaa et al. 2019).

All the above promising results were achieved using viral vectors as a carrier, and one of the main concerns when using viral vectors for gene therapy is induced toxicity due to overexpression of the transgene by the constitutive promotors.

Recently, very high level of FXN overexpression was reported to lead to mitochondrial dysfunction and cardiac toxicity in mice. Indeed, FXN cardiac overexpression up to ninefold of the normal endogenous level was reported to be safe, but significant heart toxicity was observed above 20-fold endogenous expression (Belbellaa et al. 2020). A recent study revealed a direct link between FXN's primary function and the AAV-mediated toxicity seen in vivo *(*Huichalaf et al. 2022*)*. The high expression of FXN mimicked the symptoms of FXN insufficiency, including a lack of Fe-S cluster-dependent activities. Biodistribution study of the AAV9-CAG-human frataxin (hFXN)-HA vector revealed a high transduction of the liver, a moderate transduction of the heart and brain, a milder transduction of the DRG, and a poor transduction of the spinal cord and cerebellum (Piguet et al. 2018). Thus, understanding the threshold for safe FXN gene expression level is a key to avoid cardiac toxicity or neurotoxicity. All the available studies and data points to the necessity of refining the expression levels of transgene optimize the usage of appropriate viral serotype and administration mechanism. The first step of refining the methods would involve the choice of adapted promoter for gene transcriptional regulation, which can be either ubiquitous or cell-type specific.

In parallel to the advances in treating the cardiomyopathy, it was also important to develop an appropriate model for exploring and testing treatment options to reduce or reverse the ganglionopathy and sensory neuropathy associated with FXN deficiency. A novel conditional mouse model that recapitulates the sensory ataxia and neuropathy associated with FA, but with a more fast and severe course was developed (Piguet et al. 2018). In this model, the expression of Cre-recombinase was driven under Parvalbumin (P*valb*) promotor, which is a specific promotor expressed in proprioceptive neurons in the dorsal root ganglion, brain interneurons, and Purkinje neurons, and this helps to have conditional neuronal specific knockout of frataxin (see Tables 1, 2 and Box 1).

Using this neuronal mouse model, a study showed a rapid and full recovery of the sensory neuropathy associated with frataxin deficiency in post-symptomatic administration of frataxin-expressing AAV, giving preclinical proof-of-concept for gene therapy in treating FA neuropathy (Piguet et al. 2018). The study was performed at two different time to compare the rescue in pre-symptomatic mice (3.5 weeks old) and post-symptomatic mice (7.5 weeks old). At 3.5 weeks old mice, a single intravenous injection of AAV9- CAG-FXN-HA at a dose of 5×10^{13} genomes (vg)/kg was performed in *Pvalb* cKO mice (Piguet et al. 2018). A significant coordination improvement in treated compared to untreated *Pvalb* cKO mice was observed (Piguet et al. 2018). At 7.5 weeks old mice a combined intravenous administration of AAV9-CAG-FXN-HA at a dose of 5×10^{13} vg/kg simultaneously with intracerebral deliveries of AAVrh.10-CAG-FXN-HA 1×10^{10} vg/kg in the striatum and the cerebellar white matter to target the CNS was performed, after the onset of behavioral impairment. This was able to reverse the phenotype of these mice at the behavioral, physiological, and cellular levels within a few days (Piguet et al. 2018). Dose-response study for this mouse model is yet to be performed. In conclusion, these results demonstrate the strong potential of AAV delivery to restore frataxin expression in DRG and rescue the ganglionopathy and sensory neuropathy

Table 2 Proof-of-concepts using gene therapy in FA

Target organ/tissue	Vector/serotype	Administration	Dosage	Animal models	Key results	Reference
CNS	HSV1-FXN	Intraparenchymal—Brainstem	1.44×10^4 IU	NSE cKO	Motor co-ordination reversal.	Lim et al. (2007)
Cardiomyocytes	AAVrh10-CAG-hFXN-HA	Intravenous	5.4×10^{13} vg/kg	*MCK cKO* (3.5 weeks) Pre-symptomatic	Prevention of cardiomyopathy and cardiac pathology at molecular and cellular levels	Perdomini et al. (2014)
Cardiomyocytes	AAVrh10-CAG-hFXN-HA	Intravenous	5.4×10^{13} vg/kg	*MCK cKO* (7.5 weeks) Post-symptomatic	Reversal of cardiomyopathy	Perdomini et al. (2014)
Cardiomyocytes	AAVrh10-CAG-hFXN-HA	Intravenous	2.5×10^{13} vg/kg (Dose-response study)	*MCK cKO* (5 weeks) Early stage of cardiac dysfunction	Only 50% transduction of cardiomyocytes, corrects the cardiac function	Belbellaa et al. (2019)
Cardiomyocytes	AAVrh10-CAG-hFXN-HA	Intravenous	2.5×10^{13} vg/kg (Dose-response study)	*MCK cKO* (7.5 weeks) Post-symptomatic	Only 50% transduction of cardiomyocytes, corrects the cardiac function	Belbellaa et al. (2019)
Proprioceptive sensory neuron	AAV-CAG-hFXN-HA	Intravenous-AAV9	5×10^{13} vg/kg	*Pvalb cKO* (3.5 weeks) Pre-symptomatic	Prevention of progressive loss of sensory defects	Piguet et al. (2018)

Proprioceptive sensory neuron, Cerebellar Purkinje cell, and Deep dentate nuclei	AAV-CAG-hFXN-HA AAVrh10-CAG-hFXN-HA	*Dual administration* Intravenous-AAV9—striatum Intracerebral-AAVrh10-Cerebellum	5×10^{13} vg/kg 1×10^{10} vg/kg	*Pvalb cKO* (7.5 weeks) Post-symptomatic	Rapid reversal and rescue of sensory ataxia	Piguet et al. (2018)
CNS	AAV9-hFXN	Intraperitoneal	6×10^{11} v.p. to 6×10^{9} v.p.	NSE cKO	Increased life and improved cardiac systolic function	Gérard et al. (2014)
Cardiomyocytes	AAV9-hFXN	Intravenous	6×10^{11} v.p	*MCK cKO*	Increased life and decreased heart hypertrophy	Gérard et al. (2014)
Cardiomyocytes	AAVrh.10hFXN	Intravenous	10^{11}genome copies	αMyhc mice—stress-induced heart phenotype	Restoration of cardiac function.	Salami et al. (2020)

associated to frataxin deficiency, even in severely affected animals. While this is encouraging for the development of a therapeutic approach in clinical settings, the FA neuropathology in humans is complex, and the status of proprioceptive neurons in FA patients in the early stages of the disease remains to be determined. Mice still developed a cerebral phenotype; however, this is not a phenotype occurring in FA patients, although it is important to target the Purkinje cell and the dentate nucleus in the cerebellum of FA patients. To improve the therapeutical approach, it would be of interest to optimize overall brain transduction, especially the cerebellum, with new generation of AAV vectors with an optimized capsid, such as the newly described PHPeB vectors (Chan et al. 2017).

Interesting studies on frataxin transcriptional regulation has defined the minimal regions required for an efficient FXN expression, including sequences from the 5′UTR and the first intron of the gene (Li et al. 2020). In the same study, these sequences have been used to produce different mini-*FXN* genes, with the goal of developing gene therapy vectors that produce close to endogenous frataxin expression. In vitro experiments demonstrate that these minigenes have an efficient expression in HEK293 cells, but also in induced pluripotent stem cells-derived neurons and cardiomyocytes, after AAV-mediated transduction (Li et al. 2020). Proof-of-concept studies need to be further developed with these new expression cassettes. Additionally, use of regulatory sequences, such as introns, enhancers, silencers, polyadenylation sequences, and insulator elements could allow a finer tune of the dose when it comes to dosages of vectors used in therapeutics (Ingusci et al. 2019).

Gene therapy is one of the most promising approaches for the treatment and cure of rare hereditary diseases such as FA. However, given the fact that FA is a multisystemic disorder, targeting all affected cell types with a single vector would be difficult to achieve, specifically with the current vectors. In addition, with the existing vector manufacturing and delivery methods, the biodistribution efficiency achieved in pre-clinical studies are unlikely to be replicated in the clinical trials. As described, another major difficulty is to express and maintain a safe level of frataxin in transduced cells, which will depend on the future development of vector expressing frataxin with its endogenous promoter. At this date, more pre-clinical work needs to be performed to develop the adequate expression cassette, the vectors and the delivery approaches for gene therapy. Therapeutic impact will in FA presumably depend on the stage of disease when the gene therapy is administered. Finally, it will be impossible to treat all affected tissues, and gene therapy in the future will need to be combined with approaches such as pharmacological treatment described above.

5.3 Protein Replacement Therapy

Protein replacement therapy is complementary to gene therapy, and aims at delivering exogenous frataxin directly to the mitochondria to treat the cells lacking frataxin. The trans-activator of transcription (TAT) protein transduction domain is an

11-amino-acid positively charged peptide that has been proven in vitro and in vivo to draw a variety of compounds across cell membranes (Vyas et al. 2012). TAT-built fusion proteins have been demonstrated to enter and exit cells quickly (Moore and Payne 2004), as well as capable of crossing the blood brain barriers (Asoh et al. 2002; Ye et al. 2011). Therefore, the use of the TAT transduction peptide is appealing for FA. As frataxin is a mitochondrial targeted protein, the TAT-FXN fusion protein (CTI-1601) developed is proteolytically cleaved upon mitochondrial transduction, removing the TAT peptide at the same time as the MTS (Vyas et al. 2012). This has a great advantage of "locking" mature frataxin within the mitochondria. When the TAT-FXN fusion protein was injected in the cardiac conditional FA mouse model, the *Mck* cKO mice showed increased lifespan and increased in diastolic function of heart, although a complete rescue of phenotype was not observed (Vyas et al. 2012). In addition, molecular characteristics were also rescued, such as aconitase activity in the cardiac tissue. A second TAT-FXN fusion construct was tested in vitro on frataxin deficient DRG neurons to determine the rate of reversal of the neurodegeneration (Britti et al. 2018). In this study, the construct used the mitochondrial targeting sequence of citrate synthase (instead of using the FXN MTS as in the first study). TAT-csMTS-FXN showed a more efficient mitochondrial cleavage, an in vitro decrease in neurite degeneration and reduced levels of apoptotic cell markers, and were shown to be able to increase lifespan in two mouse models (Britti et al. 2018).

Currently, CTI-1601 has been granted Rare Pediatric Disease designation, Fast Track designation, and orphan drug status for FA by the U.S. Food and Drug Administration (FDA). In the Phase 1 trial, to evaluate the safety, tolerability and pharmacological properties, increasing doses were tested on randomly assigned participants by giving a single subcutaneous (under-the-skin) injection of CTI-1601(TAT-FXN). The doses range from 25, 50, or 100 mg, or a placebo. The treatment was well tolerated, with no treatment-related serious adverse side effects reported, and increased frataxin levels in participant tissues, as per the company's studies. According to a press release from Larimar, the company reported deaths in non-human primates in ongoing toxicology studies at the highest dose levels. As a result, the FDA put the study on hold until it could complete a full review of the ongoing study.

More information and follow-up on the clinical studies are available at https://larimartx.com/our-programs/cti-1601/

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Therapeutic Use of Interferon Gamma in Friedreich Ataxia

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Abstract Friedreich's ataxia (FRDA) is a progressive neurodegenerative disorder caused by GAA triplet expansion in the *FXN* gene. At the cellular level, FRDA is associated with the deficiency of frataxin, a mitochondrial protein that plays a fundamental role in iron homeostasis and in the management of oxidative stress. The disease onset is usually in adolescence, leading to progressive disability. There is still no treatment to cure or halt the disease. Over the years an increasing number of drugs have been tested targeting different parts of the pathological cascade. One of the drugs tested has been interferon-gamma (IFN-γ). IFN-γ is currently approved for the treatment of chronic granulomatous disease and severe malignant osteopetrosis. In patients with FRDA, IFN-γ upregulated frataxin levels in cells from FRDA patients and increased frataxin expression in dorsal root ganglia neurons. In this chapter we review the basic science behind the proposal of IFN-γ as a potential treatment for FRDA and summarize the clinical studies related to the use of IFN-γ in FRDA, outlining critical lessons that have been learned in terms of drug efficacy and tolerability.

Keywords Friedreich's ataxia · Interferon · Treatment

Friedreich's ataxia Friedreich ataxia (FRDA) is the most common hereditary ataxias, with a prevalence of ~1:50,000. The genetic defect consists, in most cases, of an expanded number of GAA triplets within the first intron of the autosomal *FXN* gene, resulting in epigenetic suppression of the gene itself and consequent reduced production of the protein frataxin. In its mature form, human frataxin is a ~15 kDa,

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_24

130 amino acid globular protein (Condò et al. 2007), mostly found in mitochondria. Frataxin plays an important role in cellular iron metabolism, particularly in the assembly of iron-sulfur clusters. These are functional groups contained in many enzymes, including those involved in mitochondrial metabolism and energy production. Iron-sulfur clusters are also a major storage site for intracellular iron (Castro et al. 2019; Li 2019). Frataxin is essential for survival, as complete absence of frataxin is not compatible with life of complex organisms. While in healthy heterozygous subjects frataxin levels, as measured both in buccal cells and peripheral blood cells, are at >50% of normal levels, in homozygous conditions, frataxin protein may be 10–30% of normal levels (Deutsch et al. 2010). Such low levels are insufficient for optimal mitochondrial metabolism. This results in mitochondrial dysfunction, oxidative cellular distress, and premature death of specific classes of peripheral and central neurons, particularly sensory neurons of the dorsal root ganglia (DRG) and neurons in the dentate nucleus (Delatycki and Bidichandani 2019; Harding et al. 2021). The disease has the clinical features of a chronic, unremitting and generally slowly progressing, yet eventually severe, neurological dysfunction, impairing primarily motor coordination, often associated with hypertrophic cardiomyopathy that may evolve into overt heart failure. There is no approved therapy for FRDA yet. Patients mostly rely on physical therapy and general anti-oxidative pharmacological support.

The quest for an effective therapy over the past 20 years has followed different strategies, including drug discovery, drug repurposing, gene editing or replacement, frataxin protein replacement, and mitochondrial and metabolic enhancement, generating a variety of potential therapeutic approaches currently under investigation, at different stages of pre-clinical and clinical evaluation (Clay et al. 2019; Ocana-Santero et al. 2021). Among them, a significant amount of interest, and clinical effort, has been generated by the possible therapeutic use of interferon gamma.

Interferon Gamma: A Pleiotropic Cytokine A naturally occurring cytokine, interferon gamma (IFNγ), is primarily involved in the regulation of the immune system. It is also named type II interferon, to distinguish it from type I interferons, a class of genetically and structurally unrelated cytokines. IFNγ is a ~17 kDa polypeptide released by, and acting upon, a variety of cells, particularly, but not limited to, cells of the immune system, such as most classes of lymphocytes, macrophages, and other antigen presenting cells. Notably, among the non-immune cells that might be affected by IFNγ are the DRG sensory neurons, which are both capable of releasing and responding to IFNγ, in an autocrine fashion (Neumann et al. 1997). IFNγ regulates both innate immunity and adaptive immune responses against a variety of potential pathogens, including viruses, intracellular and extracellular bacteria, fungi, protozoa, and helminths. Importantly, a major antimicrobial function of IFNγ appears to involve the control of iron trafficking and redistribution (see below). IFNγ also stimulates anti-tumor surveillance by inducing anti-proliferative states and activating NK cells. Moreover, IFNγ plays an important role in regulating cytokine microenvironment, generally skewing the immune response toward Th1 phenotypes. Finally, it also impacts the redox status of lymphoid organs and tissues,

influencing cellular homing and trafficking as well as cell division, differentiation, ageing, and death (Schroder et al. 2004).

IFNγ exerts its actions by binding to a cell membrane receptor (IFNγR), that signals through the Jak-Stat pathway. The IFNγR is a tetramer, expressed by multiple cell types, made of two ligand-binding subunits (IFNγR1) and two associated signal-transducing subunits (IFNγR2). Biologically active IFNy is a non-covalent homodimer, formed by the self-association of two polypeptides in antiparallel orientation. Upon binding of the dimeric ligand to the IFNγR1 chains, a number of phosphorylation events occur, involving different Janus Kinases (Jak1 and Jak2) recruited to both the INFγR1 and IFNγR2. This eventually leads to phosphorylation and homodimerization of the nearby cytosolic transcription factor Stat1, its dissociation from the receptor and its translocation to the nucleus. Here, Stat1 homodimers can bind to GAS (gamma activated sequences) elements within the promoter region of a variety of genes, regulating their transcription. Signaling is terminated when the IFNγ-IFNγR1 complex is internalized, enters the endosomal pathway where the complex dissociates, and the ligand is degraded. A number of cytosolic and nuclear phosphatases also negatively regulate the activation of Jaks and Stats, effectively modulating and terminating IFNγ signaling (Kak et al. 2018).

Interferon Gamma and Iron Metabolism The battle for iron control is critical in host–pathogens interactions and a major concern for innate immunity. Bioavailable iron redistribution between extracellular fluids and tissue macrophages occurs within hours from the detection of the infection, in order to prevent pathogens from accessing iron. This process is highly conserved throughout evolution and co-regulated by IFNγ. In general, IFNγ-controlled mechanisms force iron out of pathogen-infected macrophages (Abreu et al. 2020), or alternatively, allow extracellular iron to relocate inside macrophages, in case of infection by extracellular pathogens (Nairz et al. 2014). This is achieved by IFNγ-mediated modulation of the expression of key players of iron metabolism and trafficking, including ferritin, transferrin, TFR1 (transferrin receptor 1), the iron transporters NRAMP1 (natural resistance-associated macrophage protein-1) and DMT1 (divalent metal transporter 1), the regulator of intra/extracellular iron fluxes FPN1 (ferroprotein 1), its ligand and regulator HAMP (hepcidin antimicrobial peptide) and the transcription factor IRP2 (iron regulatory protein 2). Levels of intracellular iron can be therefore regulated by IFNγ at multiple levels (Nairz and Weiss 2020). Considering that most intracellular iron is associated with iron-sulfur clusters and that frataxin is a key component of the iron-sulfur cluster assembling machinery, it might not come as a surprise that IFNγ could also regulate the amount of frataxin protein levels inside living cells.

Interferon Gamma Reverses Frataxin-Deficient Phenotypes It was initially observed that IFNγ can upregulate frataxin protein in vitro in multiple cell types, including transformed cell lines or peripheral blood mononuclear cells (PBMC) freshly isolated from healthy donors (Tomassini et al. 2012). Importantly, IFNγ can

upregulate frataxin also in frataxin-deficient fibroblast cell lines derived from FRDA patients and in PBMC freshly isolated from FRDA patients. IFNγ-induced enhancement of frataxin protein levels in FRDA cells was found to be associated with a concomitant increase in frataxin mRNA, that could be blocked by actinomycin-D, suggesting a possible transcriptional mechanism. The ability of IFNγ to upregulate frataxin, and its therapeutic potential, was then tested in YG8R mice, a frataxin-deficient genetically engineered animal model for the disease, dosed subcutaneously with IFNγ, or control solution, for several weeks. IFNγ treatment was capable of delaying the appearance of the disease phenotype, ameliorating the motor coordination performances of the mice, including ambulatory distance, average velocity, vertical jumps, and latency to falling from a rotarod, compared to control solution. This was associated with an increase of frataxin expression within DRG neurons in IFNγ-treated mice, compared to control solution-dosed mice (Tomassini et al. 2012). These encouraging results prompted the evaluation of the therapeutic potential of IFNγ in FRDA patients.

IFNγ in the Clinic IFNγ has a development history spanning over three decades. The only IFNγ approved for clinical use is a recombinant human version. Recombinant human IFNγ (interferon gamma 1b, IFNγ1b, trade name Actimmune in the United States, and Imukin in the European Union) is a biologically active, 140 aa-long mature polypeptide, deleted of the signal peptide, formulated for subcutaneous injection. The therapeutic potential of IFNγ1b has been investigated in a variety of infectious diseases, autoimmune diseases, immunodeficiencies and cancers. IFNγ1b has been FDA approved in 1991 for the treatment of chronic granulomatous disease, a rare pediatric genetic autosomal disease affecting granulocytes and causing multiple bacterial infections, and in 2000 for the treatment of severe malignant osteopetrosis, a rare pediatric genetic autosomal disease affecting bones. IFNγ1b had previously obtained orphan drug designation by the FDA for both indications, in 1988 and in 1996 respectively. Its proven effect associated with an overall favorable safety profile and approval for the chronic treatment of pediatric diseases, made IFNγ1b (Imukin) a good candidate for testing in FRDA patients.

1 Interferon Gamma in FRDA Clinical Trials

With the advent of the pre-clinical in vitro and in vivo results of IFNγ in FRDA patients' cells and FRDA YG8R mice (Tomassini et al. 2012), a wide road was paved into testing this drug in patients by means of clinical trials. Accordingly, several trials were designed and completed in the following years. Seven original research papers have been published over a period of approximately 5 years, from 2015 to 2020 (Table 1). These studies were conducted in Europe (n = 4, Italy, Turkey, and Norway) and in the United States (n = 2). All articles have been published in peer-reviewed journals. A complete tabulation of the included studies is provided in Table 1.

Table 1 Summary of IFNγ studies in Friedreich's ataxia

Reference	Country	Experimental design	Objectives	Protocol	Inclusion criteria	Exclusion criteria
Seyer et al. (2015)	USA	Open-label pilot study, dose-escalating, single site	Safety, tolerability, efficacy	Dose escalation: 10, 50 μg/m^2 (tot 12 weeks)	Age 8–17 yrs, genetic diagnosis	Clinically significant cardiac/hepatic/renal disease or unstable illnesses.
Marcotulli et al. (2016)	Italy	Phase IIa clinical trial, dose-escalating	Safety, tolerability, efficacy	Three escalating doses: 100, 150, 200 μg (tot 35 days)	Age 18–45 yrs, genetic diagnosis, willingness, and ability to comply with the study protocol.	Breastfeeding, pregnancy, unstable clinical condition (cardiovascular disease, renal, hepatic, hematologic, gastro-intestinal, endocrine, pulmonary, immunologic, or local active infection/infectious illness, transplanted organ, psychiatric disease, and drug or alcohol abuse), previous use of IFNγ, participation in other clinical trials within 30 days of the screening visit.
Wyller et al. (2016)	Norway	Case report	Efficacy in severe cardiomyopathy	Dose escalation: 10, 100, 150 μg (tot 52 weeks)	n.a.	n.a.

Table 1 (continued)

Reference	Country	Experimental design	Objectives	Protocol	Inclusion criteria	Exclusion criteria
Lynch et al. (2019)	USA	Randomized, double-blind, placebo-controlled, dose-escalation, multicenter study	Efficacy, safety, pharmacokinetics	*Double blind phase*: Randomization 1:1 to receive either IFN-γ 1b or matching placebo: dose escalation 10, 25, 50, 100 μg/m^2 (tot 26 weeks). *Open-label extension study* (6 months)	Age 10–25 yrs, GAA biallelic expansion, functional stage of >1 to <5, ability to walk 25 feet with/out an assistive device.	History of substance abuse, clinically significant cardiac disease, hypersensitivity to IFNγ or *E. coli*-derived products, moderate/severe renal, or hepatic disease, significant abnormalities of white blood cell count, hemoglobin, or platelet count.
Vavla et al. (2020a, b)	Italy	Open-label phase II, single-center, longitudinal, one-step dose-escalation	Safety, efficacy	Dose escalation: 100, 200 μg (tot 6 months), follow-up (6 months)	Age 10–40 yrs, genetic diagnosis, pre-study assessment	Concurrent unstable medical condition (cardiac, respiratory, liver, kidney failure), myelosuppression, blood dyscrasia, hypersensitivity to IFNγ/latex, exposure to EPO within 3 yrs prior to study initiation, use of other medications intended for FRDA, pregnancy, breastfeeding.
Yetkİn and GÜltekİn (2020)	Turkey	Retrospective study	Efficacy, tolerability, prognosis.	Dose: 50 μg	Age 25–40 yrs, biallelic GAA expansion, ambulatory patients.	No point mutations, refusal of treatment, unstable clinical condition due to a chronic disease, liver disease, pregnancy, breastfeeding.

Seyer et al. (2015)	Frataxin protein/ mRNA in whole blood/ buccal cells. FARS, PedsQL, ADL, MFIS.	12 (3/9)	12	n.r.	6	835	n.r.	Common AEs, SAEs $n = 2$	Well tolerated. Slight increase in RBC frataxin levels. Clinical 5 point FARS score and upper extremities improvement.
Marcotulli et al. (2016)	Frataxin levels in PBMC. SARA, EcoCG, EKG	9 (6/3)	Range 21–38	n.r.	16.44 + 5.174	Range 100–1350	Range 216–1350	Common AEs	Well tolerated. No changes in frataxin levels overall and in neurological scores.

(continued)

Table 1 (continued)

Reference	Efficacy outcome measures	FRDA sample size (F/M)	Age (mean ± SD, range in yrs)	DD (mean ± SD, range in yrs)	AAO (mean ± SD, range in yrs)	GGA 1	GAA 2	Side effects	Results
Wyller et al. (2016)	EcoCG, EKG (Sokolow-Lyon index), Troponin T, NT-proBNP, MMP-9. Frataxin mRNA levels in whole blood. FARS, QoL.	1 (F)	18	9	9	700	700	Common AEs	Decrease in troponin T/ NT-proBNP/ Sokolow index; improvement in frataxin levels/ markers of diastolic function/ cardiac remodeling/ quality of life. No changes in FARS score.
Lynch et al. (2019)	mFARS, frataxin protein levels in whole blood/ muscle biopsies/ buccal cells; Ataxia Staging, ADL, MFIS, PedsQL, SF-36.	Double-blind phase 92: 47 IFNγ arm (F 55.3%), 45 placebo (F 57.8%); Open label extension started 86, completed 51.	IFNγ arm 16.5 ± 4.4, placebo arm 16.1 ± 3.8	IFNγ arm 11.9 ± 4.1, placebo arm 12.2 ± 4.1	IFNγ arm 9.5 ± 3.9, placebo arm 9.1 ± 3.8	IFNγ arm 706 + 166, placebo arm 711 + 224	n.r.	Common AEs. Double-blind phase $n = 4$, Discontinuation $n = 1$; Extension open-label SAEs n = 9, Discontinuation $n = 31$, Death $n = 1$.	During the double-blind phase no changes in mFARS/ frataxin levels. During the open-label extension period, a more stable course of disease.

Vavla et al. (2020a, b)	SARA, EchoCG, EKG (Sokolow-Lyon index), Troponin I, frataxin protein in PBMC, OCT, DTI, fMRI.	12 (5/7)	17.33 ± 4.54 (11–26)	8.83 ± 4.59 (2–15)	8.5 ± 3.18 (4–14)	697.33 ± 123.92 (460–862)	942.83 ± 141.94 (750–1166)	Common AEs, SAEs $n = 2$, Discontinuation $n = 1$.	Halt in SARA score progression, reduced IVS and Sokolow-Lyon index, rebound effect during follow-up. No changes in frataxin levels/ OCT. DTI changes in 12 months period. motor task and RS-fMRI changes, inverse correlations between SARA scores and functional activity in the R M1 fMRI activation for both.

(continued)

Table 1 (continued)

Reference	Efficacy outcome measures	FRDA sample size (F/M)	Age (mean ± SD, range in yrs)	DD (mean ± SD, range in yrs)	AAO (mean ± SD, range in yrs)	GGA 1	GAA 2	Side effects	Results
Yetkİn and GÜltekİn (2020)	SARA	14 (10/4)	28.92 ± 5.69	11.50 ± 5.11	n.r.	n.r.	n.r.	Common AEs, SAEs *n* = 1, Discontinuation *n* = 3.	SARA gait changes at 3 months and gait/stance at 6 months.

Legend: *FRDA* Friedreich's ataxia, *n.r.* not reported, *n.a.* not applicable, *IFNγ* interferon gamma-1b, *EPO* erythropoietin, *F* female, *M* male, *SD* standard deviation, *yrs* years, *DD* disease duration, *AAO* age at onset, *GAA1/GAA2* short/long GAA triplet expansion, *AE* adverse event, *SAEs* severe adverse events, *FARS* Friedreich ataxia rating scale, *SARA* the scale for the assessment and rating of ataxia, *PedsQL* pediatrics quality of life, *ADL* activities of daily living, *MFIS* modified fatigue impact scale, *RBC* red blood cells, *PBMC* peripheral blood mononuclear cells, *EchoCG* echocardiography, *EKG* electrocardiography, *pro-BNP* pro-brain natriuretic peptide, *MMP-9* matrix metallopeptidase 9, *QoL* quality of life, *SF-36* 36-item short-form health survey, *OCT* optical coherence tomography, *DTI* diffusion tensor imaging, *fMRI* functional magnetic resonance imaging, *RS-fMRI* resting-state fMRI

1.1 The Design of Studies

Study design varied among the different trials. One study was designed as randomized, double-blind, placebo controlled and multicenter (Lynch et al. 2019) while the remaining ones are a case report (Wyller et al. 2016), three open label phase II dose-escalating monocentric studies (Seyer et al. 2015; Marcotulli et al. 2016; Vavla et al. 2020a, b) and a retrospective study (YetkİN and GÜltekİn 2020). The study reported by Vavla et al. will be described in detail in the following section.

All these studies aimed to explore the safety of IFNγ in FRDA cohorts by monitoring and recording adverse events, tolerability, pharmacokinetics, as well as looking at its efficacy profile. The case report describes a patient with severe cardiomyopathy in whom, given the lack of effective treatments available IFNγ was provided as a last resource approach with no other expectations.

1.2 Protocols, Primary and secondary outcome meaasures of Safety and Efficacy

In almost all the studies but one the same regimen of subcutaneous injections three time per week was used. One study tested instead the effect of three escalating doses of IFNγ injections over 35 days in nine patients (Marcotulli et al. 2016). The treatment duration varied among studies, from 12 to 52 weeks. The dosage varied from 10 to 200 μg, and all studies used the dose escalation modality.

All the studies utilized specific inclusion criteria such as genetic diagnosis, and considered a specific age range including childhood (Seyer et al. 2015), adulthood (Marcotulli et al. 2016) and both children and young adults (Lynch et al. 2019; Vavla et al. 2020a, b). Disease severity was considered as an inclusion criterion in two studies that required the preserved ability to walk (Lynch et al. 2019; YetkİN and GÜltekİn 2020).

All these studies had similar exclusion criteria such as clinically unstable systemic illnesses, previous exposure to IFNγ or erythropoietin, hypersensitivity to IFNγ, pregnancy, and breastfeeding.

The assessment protocols for safety included the annotation of adverse events (AEs) and blood tests monitoring. On the other hand, the efficacy outcomes consisted in a wide range of indicators such as clinical measures (various ataxia severity scales), frataxin levels (assessed in blood, buccal cells, and muscle biopsy), cardiac measures (echocardiography, EKG, troponin levels, NT-proBNP), neuroimaging measures (fMRI), retinal measures (optical coherence tomography), and questionnaires for the quality of life and disability.

Overall, data from 140 FRDA patients were analyzed, with sample size ranging from 1 to 92 patients. All studies with the exception of Lynch et al. (2019) that tested IFNγ efficacy in a cohort of 92 patients, involved a small number of patients (<15).

Sex was reported in nearly all studies, with numbers reflecting a sex representation that was not always balanced within the same study.

Diagnosis was based on Harding's criteria and confirmed by molecular tests on genomic DNA from peripheral blood. Only one study failed to report the GAA triplet size of both the small (GAA1) and long (GAA2) allele (YetkİN and GÜltekİn 2020). All but three studies (Lynch et al. 2019; YetkİN and GÜltekİn 2020; Seyer et al. 2015), reported the GAA size of both alleles. The size of the GAA1 and GAA2 alleles ranged from 100 to 1350 triplets.

While Lynch et al. (2019) considered subjects with an FRDA functional stage of >1 to <5, and required the ability to walk 25 feet with or without an assistive device, a similar retained function was not requested in the other studies. Disease severity as measured by ataxia scales was reported as SARA scale mean score of 26.55 (Marcotulli et al. 2016), and 18.46 (Vavla et al. 2020a) and by FARS scale mean score of 69.5 (Seyer et al. 2015), or >50 (Wyller et al. 2016; Lynch et al. 2019). Disease severity is perhaps difficult to compare among the studies as they used two different scales.

All studies reported common AEs and some severe adverse events (SAEs): one in the study by YetkİN and GÜltekİn 2020, two in the studies of Seyer and Vavla, four during the double-blind, and nine during the open-label extension phase of the large study by Lynch et al. Instances of withdrawal were reported, but were not associated to the drug used in the study (Lynch et al. 2019; Vavla et al. 2020a; YetkİN and GÜltekİn 2020). Only one death was recorded during an open label extension phase, reported as not related to the study drug (Lynch et al. 2019).

Efficacy data were overall controversial. The open studies reported mild efficacy (Seyer et al. 2015) with a reduction in FARS score of 5 points, a stable disease severity with evidence of SARA progression in the follow-up (Vavla et al. 2020a) and SARA gait improvements at 3 and 6 months from treatment initiation (YetkİN and GÜltekİn 2020). The remaining studies did not report any significant variation in the disease severity scores.

Heart-related measures were relevant in the case report (Wyller et al. 2016) and in the open label study (Vavla et al. 2020a).

Frataxin levels were reported to be slightly increased in the first published study (Seyer et al. 2015), but none of the following studies reported any significant variation in the protein levels. Frataxin was assessed systematically on peripheral mononucleated blood cells, a tissue with low and possibly variable expression levels of frataxin. Occasional evaluation on muscle biopsy (Lynch et al. 2019) showed increased frataxin levels in treated patients, but muscle biopsy was not routinely included in any trial, thus the finding has only anecdotal value.

Except for the study by Lynch et al. (2019) which was designed as a double-blind placebo-controlled study thus qualifying as gold standard for testing drug efficacy, the remaining studies were all open label, therefore results should be considered cautiously. Although the study by Lynch et al. presented the largest FRDA cohort so far exposed to IFNγ, and reported lack of treatment efficacy, some caveats have been signaled by the authors themselves. The first potential problem was the heterogeneous assessment methodology associated with the multicenter nature of the

study: the multiplicity of recruitment stations while assuring the required number of participants, necessarily involved multiple raters. This was associated with a wide variation in FARS scoring well above the expected inter-rater variability. A second critical element was the timing of the procedures. To include all procedures in 1 day and still allow sufficient post-injection observation time, the drug was administered in the morning, and the efficacy assessments were performed at 4–6 hours after dosing. This timing could have compromised the severity scale performance measures as the scoring could have been affected by the mild but very frequent AEs (malaise, flu-like symptoms, headache) that commonly appear in >80% of patients after the drug administration. Interestingly, in the open label extension of the study, patients that received IFNγ for 52 weeks showed a more stable disease course, compared to natural history data.

In conclusion, the published studies report convergent data regarding the safety of IFNγ in FRDA with few and often not drug related severe adverse events and frequent, but well tolerable mild adverse events. Conclusion regarding efficacy from open studies is encouraging, but the results from the only double-blind placebo-controlled study point toward a lack of efficacy on common clinical measures and therefore one might conclude for a proven lack of efficacy of the drug. However, such conclusion needs to be taken with great caution since the data are not sufficient when considering the methodological aspects discussed above. Comparison among different studies is further hampered by the heterogeneity in the design and the chosen end points. No study lasted more than 6 months, thus no data are available on the long-term effect of treatment.

However, the analysis of the published studies gives some useful indications for future trials.

One key factor to be considered is the rate of progression of the disease: progression is not the same in the different age groups. In particular, it has been observed that the rate of progression is higher in young subjects (Patel et al. 2016; Reetz et al. 2016). This element is fundamental in the design of a clinical study. When measuring the effectiveness of placebo-controlled clinical trials for FRDA, the clinical scales used to quantify the disease status are usually characterized by poor sensitivity, given the individual variability and different natural history. The quite simple FARS-ADL scale has been recently shown to perform better than the commonly used symptom related scales (Reetz et al. 2021), but none of the reported IFNγ studies included the FARS-ADL as efficacy measure.

More specific and objective parametric measures might also improve the power of the trials. Studies by Wyller and Valva have shown that IFNγ in FRDA can modify cardiac parameters. Myocardial involvement in FRDA doesn't correlate always with neurologic impairment, but it should not be forgotten that hypertrophic cardiomyopathy ultimately resulting in heart failure is the leading cause of death in these patients. Monitoring of cardiac parameters represents a fundamental element for the patient's survival, although the significance of the short-term observed changes in morphometric parameters might not be straightforward.

Extending the assessment to a pre- and post-treatment observation period to accommodate for the natural progression of the disease might offer a more

individualized perspective on functional changes. It is important to focus on young subjects, even though the evaluation of children may prove a methodological challenge. In subjects with short disease duration even minimal variations in the rate of progression may lead to significant clinical variations over time.

Finally, it would be useful to assess long-term efficacy of INFγ in FRDA, with consistent protocols that are sensible to even minimal clinical and laboratory changes. A long-term study or a "run-in" pre-trial period of drug exposure would allow a clearer separation between responders and non-responders.

1.3 Open Label Trial of High Dose IFNγ with Objective Indicators of Efficacy

Friedreich's ataxia is still without an effective treatment. Several studies have explored the safety and efficacy of IFNγ in this disease using clinically based endpoints with mixed results (see previous section).

The coarse nature of the available clinical assessment tools for FRDA implies the need of a very high number of patients to achieve sufficient power if using them as primary end points (Reetz et al. 2016, 2021). Moreover, given the uneven progression rates observed frequently among young FRDA patients, subtle changes in disease progression may result in high variability even over relatively short times (months). We conducted recently an open-label one step dose escalation phase II study in which clinical indicators were coupled to objective paraclinical measures. Leaving aside the encouraging results of the trial and its main weakness, that is the open design, we believe its description could be useful in view of a possible reconsideration of IFNγ as therapeutic option for FRDA. To better understand the dynamics of disease progression, and with the aim of adjusting for individual differences, the study design was prospectively organized with two non-interventional observation periods flanking the 6-month exposure to the drug (Fig. 1).

Twelve patients were recruited and 11 completed the study. The demographic and clinical baseline characteristics of the recruited patients are shown in Table 2.

The treatment with IFNγ on Dose 1 (100 μg × 3 times/week) was generally safe in all the patients with one severe adverse event that resolved spontaneously; common mild IFNγ adverse events are summarized in Table 3. The SARA scores (Fig. 2a), chosen as primary efficacy endpoints, increased during the pre-treatment observation time and showed no or slightly negative changes during the IFNγ treatment. The SARA score resumed a moderate (non-significant) uptrend after the discontinuation of the treatment (Fig. 2b). The progression of SARA score pre-treatment significantly differed from the progression during the treatment period ($p < 0.001$). When considered one by one (Fig. 2a), it is apparent that while most (eight) of the patients show a decrease in SARA score after treatment, two of them show an increase in SARA score that is milder compared to the period T −6 to T0, and one patient (Patient 10) shows a steady increase.

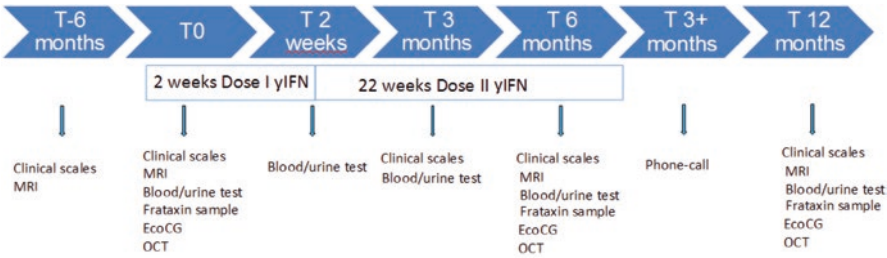

Fig. 1 Study visits and clinical-instrumental assessments performed during every visit. Dose 1 (100 μg, 3 times/week for 2 weeks), Dose 2 (200 μg, 3 times/week for 22 weeks). SARA: Scale for the assessment and rating of ataxia (Schmitz-Hubsch et al. 2006) MRI: magnetic resonance imaging. EchoCG: echocardiography, ECG: electrocardiography

Table 2 Demographic and clinical indicators at baseline: mean ± SD and range (min; max)

Variable	Baseline mean ± SD	Range: min; max
GAA1	697.33 ± 123.92	460; 862
GAA2	942.83 ± 141.94	750; 1166
Scholarity (y)	11.58 ± 3.6	6; 18
Sex (M/F)	7M/5F	
Age at onset (y)	8.5 ± 3.18	4; 14
Age at T0 (y)	17.33 ± 4.54	11; 26
Disease duration (y)	8.83 ± 4.59	2; 15
SARA at T0 (0–40)	18.46 ± 6.36	7.5; 29.5

SARA scale for the assessment and rating of ataxias, *M* male, *F* female

No significant changes were recorded in disability and quality of life measures.

Indicators of cardiac structure and function were significantly impacted by the treatment, with a reduction in the end-diastolic interventricular septal wall thickness (IVST) observed at the end of the treatment period (Fig. 3a) that returned to pre-treatment levels 6 months after the termination of the treatment (T12) (Fig. 3b). The EKG Sokolov index was also reduced by treatment and rebounded after treatment discontinuation (Fig. 4).

Although we detected a >30% increase of frataxin levels in response to the treatment in 3 out of 11 patients, no significant changes from basal levels could be observed when considering the whole group (Table 4).

No significant changes were found when considering the average retinal nerve fiber layer (RNFL) thickness for both eyes. However, a slight (not significant) general decline in retinal thickness was observed.

White matter analysis by magnetic resonance imaging (MRI) revealed a reduction of fractional anisotropy (FA) values in the left superior cerebellar peduncle at T12 compared to T0 (Table 5).

The motor-task functional MRI (fMRI) showed an increased activation of the left primary motor cortex during the movement of the dominant (right) hand ($p = 0.008$)

Table 3 Number of patients (n = 12; %) reporting the adverse events (AE) during treatment

	Dose 1		Dose 2	
AE	*n*	%	*n*	%
Headache	4	33.33	10	83.33
Fatigue	4	33.33	6	50.00
Fever	1	8.33	6	50.00
Shivers	0	0.00	5	41.67
Pain at the injection site	2	16.67	4	33.33
Flu-like symptoms	0	0.00	4	33.33
Reduced appetite	2	16.67	3	25.00
Nausea	1	8.33	2	16.67
Myalgia	1	8.33	0	0.00
Diarrhea	1	8.33	0	0.00
Vomit	0	0.00	2	16.67
Sinus congestion	0	0.00	2	16.67
Abdominal pain	0	0.00	2	16.67
Epistaxis	0	0.00	2	16.67
Bruise at the injection site	0	0.00	2	16.67
Injection site rash	0	0.00	0	0.00
Skin discoloration	0	0.00	1	8.33
Injection site swelling	0	0.00	0	0.00
Depression	0	0.00	1	8.33

Dose 1 (100 µg/3 times/week) and Dose 2 (200 µg/3 times/week)

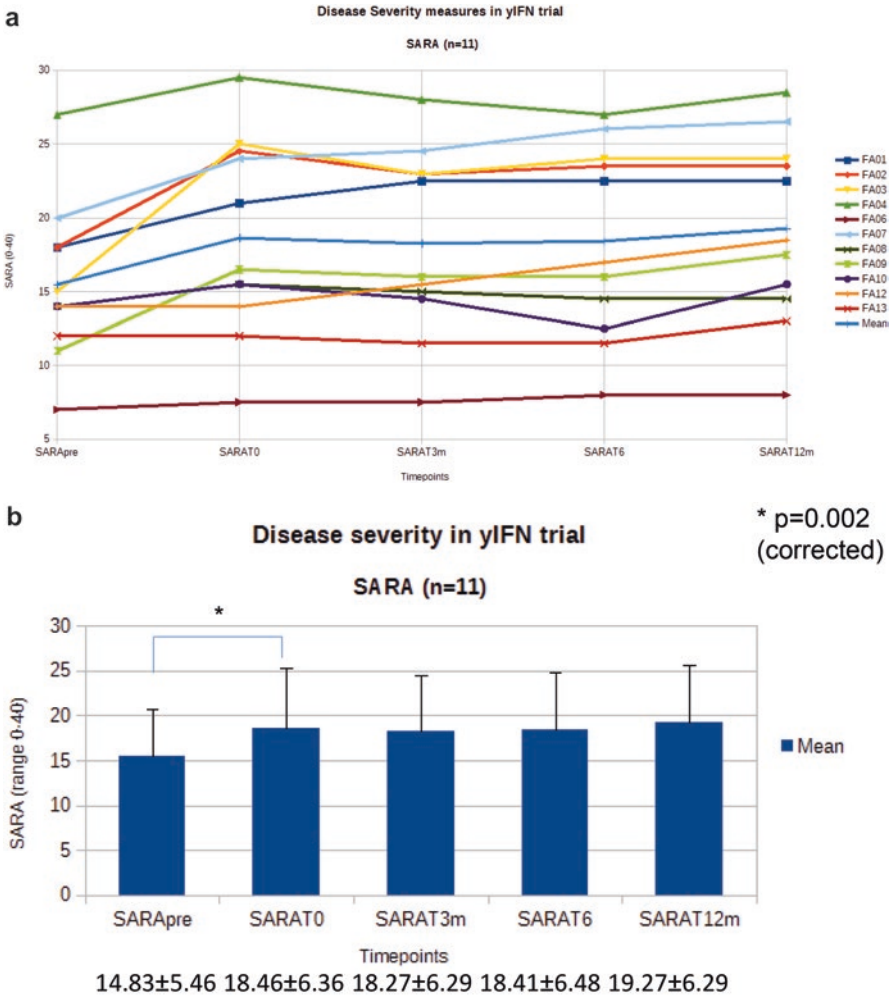

Fig. 2 Scores at the SARA of the individual subjects and their mean (FA, mean) (**a**) and expressed as mean ± SD (**b**). The score shows in almost all subjects an increase during the non-treatment observation period (Tpre-T0) and a subsequent stabilization with even a mild decrease seen in some patient

after treatment. Three resting state networks showed significantly increased activity between T0 and T12. There was a significant negative correlation between the changes of SARA scores and functional activity in the motor cortex.

The results of this clinical trial are encouraging even when considering the fundamental weakness of its open-label design. The trial objectives were to test the safety and efficacy of a high dose (200 μg, 3 times/week) of IFNγ. The treatment was safe and reasonably well tolerated with frequent occurrence (up to 83.3%) of common AE (mostly known to be associated with IFNγ treatment).

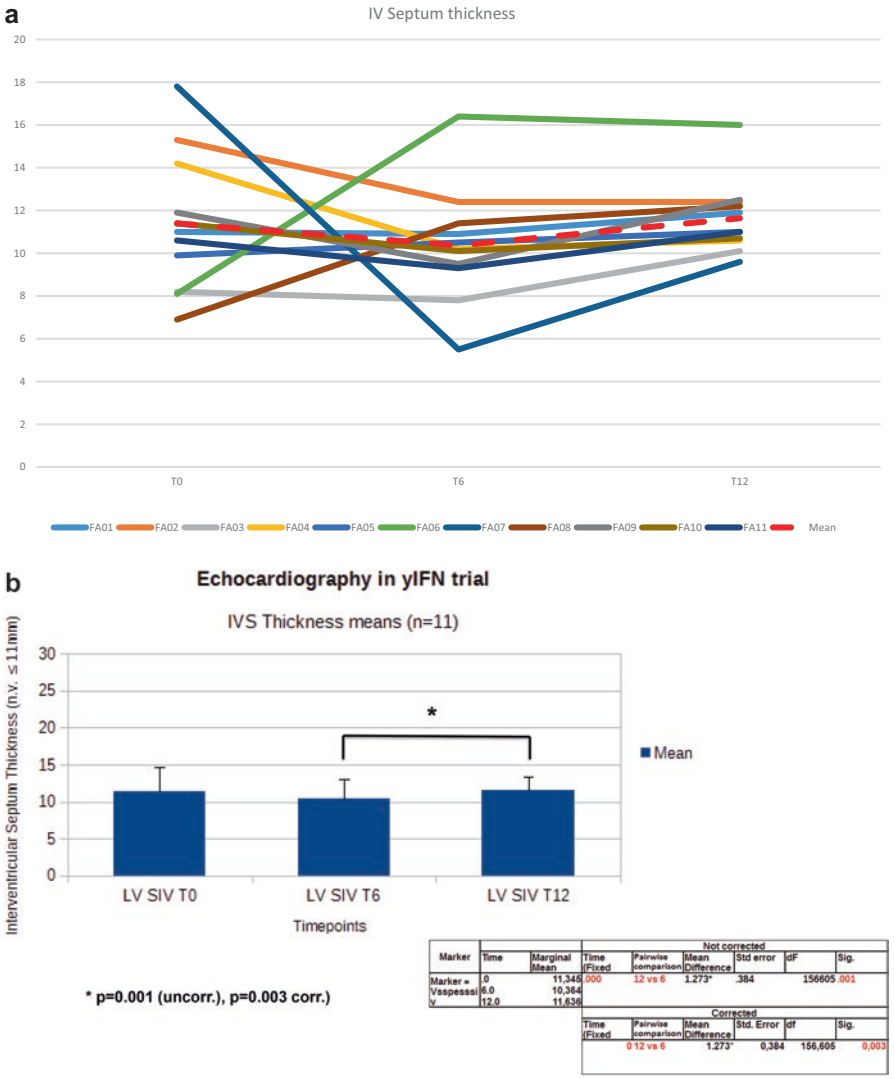

Fig. 3 Interventricular septum thickness (mm) measured by echocardiography (normal values <11 mm) at T0, T6, and T12. Data are shown for individual subject (**a**) and a mean (FA, mean) and as mean ± SD (**b**). With the exception of 2 subjects all the others show reduction of the septum thickness which in most cases rebounds at 12 months

Efficacy was assessed not only with the clinical measure (SARA), but also with a comprehensive set of monitors covering different systems and dimensions. Some of the measures showed the progressive impairment characterizing the disease, some showed the ability to capture, even in the short time of the trial duration, modifications that point to the possible efficacy of IFNγ in modifying FRDA disease course.

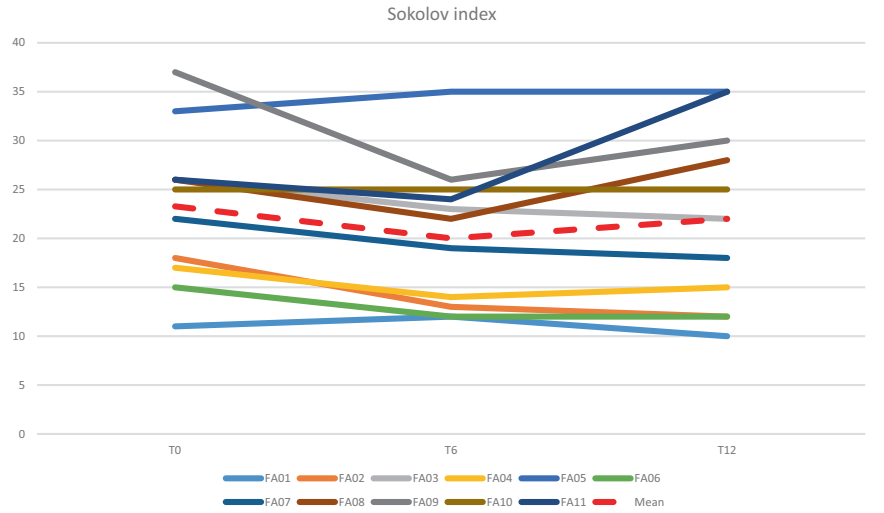

Fig. 4 Sokolov-Lyon index extracted from EKG (S in V1 + R in V5" and S in V1 + R in V6) shown for individual subject and as mean at T0, T6, and T12. A reduction in most subjects is apparent after the treatment period, which rebounds in the post treatment evaluation

Table 4 Frataxin protein was quantitated, by Western blot followed by densitometric analysis of the bands, in cell lysates from patients' peripheral blood mononuclear cells (PBMC) before the start of the treatment (T0), after three (T3), and after 6 months of treatment (T6) and 6 months after the termination of the treatment (T12)

	T0	T3	T6	T12
Frataxin	1.00	1.04 ± 0.06	1.01 ± 0.10	1.01 ± 0.11

Mean ± SEM increase at T3, T6, and T12 compared to T0, set at a value of 1, is reported for the group of 11 patients. Changes between time points were not significant

Table 5 DTI data quantitating the mean fractional anisotropy (FA) in the left superior cerebellar peduncle (LSCP) at the different time points

LSCP	T −6	T0	T6	T12
Mean FA (μ^2/s)	0.431 ± 0.014	0.440 ± 0.010	0.429 ± 0.011	0.423 ± 0.099

A general decrease was observed over time and a significant difference was measured between T0 and T12 (p = 0.019, corrected). Other comparisons were not significant. Mean FA measured in other brain regions did not show significant changes over the different time points

SARA documented the expected disease progression in the pre-treatment period, while during treatment disease progression stalled and even reversed in 8 out of 11 patients to resume progression after treatment discontinuation. The difference in SARA progression was significant, even though the one-by-one analysis of scores allows the identification of two patients who responded poorly and one who didn't respond at all. The presence of responders and non-responders is very relevant, especially when dealing with biological drugs, and has been reported frequently for

approved therapies such as disease modifying drugs in multiple sclerosis (Prosperini et al. 2017).

The lack of changes in the measures of perceived disability and quality of life are not surprising given the duration of the study and the coarse granularity of both the SF36 (Apolone & Mosconi 1998) and the WHO-DAS 2.0 (Ustun et al. 2010).

Conversely, the structural and functional evaluation of the heart through echocardiography and electrocardiography showed some significant difference after treatment. Interventricular septum thickness, an index of cardiac hypertrophy typically seen in FRDA patients with ejection fraction still above 50% (Weidemann et al. 2012) decreased after treatment and showed a sharp rebound after treatment discontinuation. Also, the Sokolow index, an indirect electrocardiographic indicator of left ventricular hypertrophy (LVH) was reduced during the treatment period and rebounded after the discontinuation of the treatment.

A RNFL thickness reduction which correlated with disease duration and age at onset has been described in FRDA patients (Seyer et al. 2013; Dag et al. 2014) but this indicator was not sensible to disease progression over 1 year time.

Structural indicators of the central nervous system such as DTI of the white matter of the brain and RNFL confirmed the widespread degeneration affecting unequally various areas of the central nervous system of FRDA patients. In this respect, our work confirmed previous work by us and others (Selvadurai et al. 2018). Fractional anisotropy (FA), a measure of structural integrity of white matter, didn't change over time, except for the left superior cerebellar peduncle (SCP) where FA showed a decline. SCPs are among the most affected tracts in FRDA, with up to 34% FA reduction. The FA decrease we observed may therefore reflect the natural disease progression which was not or only marginally modified by our relatively short treatment. On the other hand, the analysis of fMRI clearly points toward a reorganization of different sensory-motor networks during and after the treatment. It is interesting to note that such modifications seem to involve especially the sensory-motor network of the dominant hand. The negative correlation with the progression of the SARA score suggests that the MRI modifications we observed correspond to clinical improvements. The physiological implications of these findings require caution, as the precise evolution of the brain activation pattern during disease progression has not been firmly established yet.

A specific point of strength of this study was its novel design in which each subject is an effective control for him/herself in a condition resembling an "N of 1" design. Rare diseases such as FRDA require innovative clinical trial designs to accommodate for the small number of the available population and our attempt goes in that direction. This study design, with the much-needed addition of a placebo arm, could therefore be implemented in future interventional trials for FRDA to increase their potency.

The unequal response in both clinical and paraclinical indicators seen in this small group of patients highlights the possibility that the biological efficacy of IFNγ is not uniform in each individual. Based on clinical information coming from numerous FRDA patients who have been using IFNγ off-label (unpublished results), it could be safe to assume that 20–30% of FRDA patients may be weak or non-responders.

Future trials should take into consideration this aspect and adapt the trial design to selectively include patients who are most likely to benefit from the treatment.

An additional question might relate to the dosage of IFNγ. So far there is no indication that higher doses might provide additional benefit or turn "non-responder" patients into "responders" and no test has been conducted to explore this aspect. Therefore, additional clinical studies are required to investigate the safety and efficacy of higher doses and/or new formulations of human recombinant IFNγ, in Friedreich ataxia patients.

2 Conclusion

The repurposing of IFNγ for treatment in FRDA has received much attention in the last 5 years. The rationale for its use in FRDA is well founded experimentally but still not fully elucidated molecularly. The clinical response has been reported as positive by open trials, also by the use of objective paraclinical indicators, by isolated case reports and by long-term use in off label protocols. Similar positive effects were not confirmed by the 6-month placebo-controlled clinical trial, which unfortunately was affected by systematic errors and suboptimal statistical power. The question now is whether to put at rest this therapeutic option, or to consider these past experiences and design a new placebo-controlled RCT that incorporates the lessons learned during previous studies: the opportunities offered by objective indicators, the choice of different tissues on which to test frataxin levels (e.g., mouth swabs or urinary sediment), the consideration for possible responders and non-responders subjects, the need to consider the effect on clinical scales in the context of the individual rate of progression.

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Metabolic Treatments of Cerebellar Ataxia

Fanny Mochel

Abstract Metabolic causes of cerebellar ataxia encompass all categories of inherited metabolic diseases, i.e., accumulation and deficiency of small or complex molecules, and disorders of energy metabolism. Patients may present with chronic cerebellar or spino-cerebellar ataxia, paroxysmal episodes of cerebellar ataxia, or myoclonic ataxia. In case of chronic cerebellar ataxia, a fast and simple metabolic screening can identify etiologies that are amenable to treatment, especially vitamin E, cerebrospinal fluid (CSF) glucose (or, in some countries, the newly developed METAglut1™), plasma cholestanol, very long-chain fatty acids, phytanic acid, and lysosphingomyelin-509. If magnetic resonance imaging (MRI) reveals an abnormal white matter, then the activities of arylsulfatase A and galactocerebrosidase should be measured. In case of paroxysmal episodes of cerebellar ataxia, CSF glucose (or METAglut1™), plasma lactate, pyruvate, ammonium, and amino acid chromatography are of utmost importance. Glucose transporter type 1 (Glut1) deficiency syndrome, cerebrotendinous xanthomatosis, ataxia related to vitamin E deficiency, and Refsum disease are among the most common causes of treatable cerebellar ataxia. Metabolic treatments range from dietary intervention and supplementation therapies to metabolite-lowering therapies, pharmacological chaperones, and replacement therapies.

Keywords Biomarkers · Energy metabolism · Complex lipids · Metabolic treatments

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_25

1 Introduction to Metabolic Diseases

Metabolism involves thousands of proteins, mostly enzymes, cofactors, receptors, and transporters, the deficit of which causes an inherited metabolic disease (IMD). Until recently, there was a tendency to mostly consider disorders affecting the catabolism of molecules while synthesis and transport defects were clearly underrepresented. In view of the rapid recognition of these new categories of IMD by next-generation sequencing (NGS), it is now of utmost importance to integrate them in the classification of IMD based on these biochemical categories (synthesis, remodeling, transport, catabolic, and trafficking defects) rather than on an organelle-centric approach that splits arbitrarily metabolic pathways. Therefore, we have proposed a simplified classification, based on a pathophysiological approach, to help clinicians to suspect, detect, understand, and treat IMDs that are critical (Saudubray et al. 2019).

Briefly, disorders involving *small molecules* have biomarkers and are divided into two subcategories: accumulation and deficiency. Accumulation of small molecules leads to acute or progressive postnatal "intoxication," present after a symptom-free interval, aggravated by catabolism and food intake. These treatable disorders must not be missed. Deficiency of small molecules is due to impaired synthesis of compounds distal to a block or altered transport of essential molecules. This subgroup shares many clinical characteristics with complex molecule disorders. *Complex molecules* (e.g., glycogen, sphingolipids, phospholipids, glycosaminoglycans, glycolipids) are poorly diffusible. Accumulation of complex molecules leads to postnatal progressive storage like in glycogen and lysosomal storage disorders. Many are treatable. Deficiency of complex molecules is related to the synthesis and recycling of these molecules, which take place in organelles. They may interfere with fetal development. Most present as neurodevelopmental or neurodegenerative disorders unrelated to food intake. Peroxisomal disorders, congenital disorders of glycosylation, defects of intracellular trafficking and processing, recycling of synaptic vesicles, and tRNA synthetases also belong to this category. Only few have biomarkers and are treatable. Disorders involving primarily *energy metabolism* encompass defects of membrane carriers of energetic molecules as well as cytoplasmic and mitochondrial metabolic defects. This oversimplified classification is connected to the most recent available nosology of IMD (Ferreira 2021).

2 Metabolic Forms of Cerebellar Ataxia

Metabolic causes of cerebellar ataxia encompass all categories of IMD: accumulation and deficiency of small or complex molecules, as well as disorders of energy metabolism (Table 1). Patients may present with chronic cerebellar ataxia (i.e., progressive cerebellar ataxia) or spino-cerebellar ataxia (i.e., progressive cerebellar ataxia and pyramidal involvement), paroxysmal episodes of cerebellar ataxia, or myoclonic ataxia.

Table 1 Metabolic forms of cerebellar ataxia

Diseases	Chronic cerebellar ataxia	Spino-cerebellar ataxia	Episodic or acute ataxia	Myoclonic ataxia
Energy metabolism disorders				
Respiratory chain disorders	+	+	+	+
Coenzyme Q10 deficiency	+			
GLUT1 deficiency	+	+	+	+
PDH deficiency	+		+	
Complex molecules accumulation				
Cerebrotendinous xanthomatosis	+	+		
Niemann-Pick type C	+			+
Gaucher type 3	+			+
GM2 gangliosidosis	+			
Metachromatic leukodystrophy	+	+		
Krabbe disease	+	+		
Adrenoleukodystrophy		+		
Refsum disease	+			
DBP deficiency	+			
Peroxisomal biogenesis disorder	+	+		
α-Mannosidosis	+			
Sialidosis				+
Complex molecules deficiency				
NTE pathogenic variants (SPG39)	+	+		
PLA2G6 pathogenic variants	+	+		
GBA2 pathogenic variants	+	+		
B4GALNT1 pathogenic variants	+	+		
ELOVL4 pathogenic variants		+		
***ELOVL5* pathogenic variants**		+		
Cellular trafficking defect				
CDG syndrome	+	+		
Small molecules deficiency				
AVED	+	+		
Abetalipoproteinemia	+	+		
Biotinidase deficiency	+			
BBGD			+	
BVVL2	+			
Small molecules accumulation				
Urea cycle disorders			+	

(continued)

Table 1 (continued)

Diseases	Chronic cerebellar ataxia	Spino-cerebellar ataxia	Episodic or acute ataxia	Myoclonic ataxia
Mevalonate kinase deficiency	+			
L-2-hydroxyglutaric aciduria	+			

Disorders for which specific treatments are available are shown in **boldface** type
Abbreviations: *AVED* ataxia with isolated vitamin E deficiency, *BBGD* biotin-thiamine-responsive basal ganglia disease, *BVVL2* Brown-Vialetto-Van Laere syndrome 2, *CDG* congenital disorders of glycosylation, *DBP* D-bi-functional protein, *PDH* pyruvate dehydrogenase

The most common group of cerebellar ataxias related to energy metabolism is represented by oxidative phosphorylation (OXPHOS) deficiencies, especially pathogenic variants of the mitochondrial DNA and POLG pathogenic variants. OXPHOS deficiencies are not yet amenable to treatments except for defects of coenzyme Q10 (CoQ10) synthesis. Other treatable energetic disorders associated with cerebellar ataxia comprise glucose transporter type 1 deficiency syndrome (Glut1-DS) and, to some extent, pyruvate dehydrogenase (PDH) deficiency—see below, Sect. 3.1. Cerebellar ataxias related to deficiencies of small molecules are also often treatable, especially vitamin E or vitamin B (B1, B2, and B8). However, a large category of cerebellar ataxias caused by accumulation or deficiency of complex lipids (Fig. 1) are not treatable, with the remarkable exception of cerebrotendinous xanthomatosis (CTX) and Refsum disease.

3 Metabolic Treatments of Cerebellar Ataxia

Traditionally, treatments of IMD involve (i) supplementing a compound whose synthesis or import is deficient, (ii) lowering a compound whose accumulation is toxic, and (iii) increasing the residual activity of a deficient enzyme or replacing the enzyme. These various therapeutic approaches have been applied to cerebellar ataxias once the metabolic defect was identified (Table 2). These therapies are closely related to our understanding of the physiopathology (Table 2).

3.1 Dietary Intervention

Glucose transporter type 1 deficiency syndrome (Glut1-DS) is caused by impaired glucose transport across the blood-brain barrier and into glial cells due to heterozygous, mostly de novo, pathogenic variants in the *SLC2A1* gene encoding the glucose transporter GLUT1(De Vivo et al. 1991). GLUT1 is a membrane-bound glycoprotein that is particularly abundant in human erythrocytes, and brain endothelial and

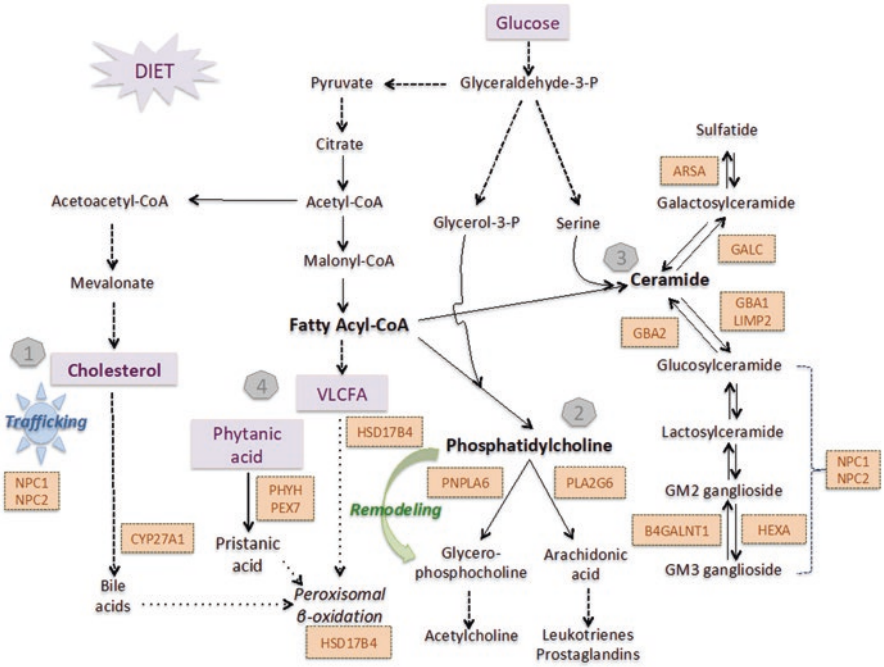

Fig. 1 Ataxia genes involved in complex lipid metabolism. Four main categories of lipids are involved in cerebellar ataxias: (1) sterol lipids, (2) phospholipids, (3) sphingolipids, and (4) fatty acids and prenol lipids. Metabolites that can also originate from the diet are indicated in purple. (From: Synofzik et al. (2019))

glial cells. Its dysfunction limits brain glucose availability and leads to brain energy deficiency. The phenotype typically comprises psychomotor retardation and permanent motor disorders (e.g., cerebellar ataxia, dystonia), associated with paroxysmal manifestations including seizures and non-epileptic paroxysmal episodes (Leen et al. 2010). With age, seizures tend to become less prominent whereas the frequency of movement disorders increases (Gras et al. 2014). The diagnosis of Glut1-DS relies on an invasive test, i.e., a lumbar puncture to measure glycorrhachia, and, sometimes complex, molecular analyses (cf. variants of unknown significance, intronic variants, mosaicism) of the *SLC2A1* gene. Likewise, we have developed a simple blood test that quantifies GLUT1 at the erythrocyte surface, METAglut1™ (Gras et al. 2017), which is 100% specific for Glut1-DS and 80% sensitive—similar sensitivity to glycorrhachia but greater specificity. Ketogenic diets, which provide ketone bodies to the brain and compensate for the lack of glucose, represent the standard of care in Glut1-DS (Klepper and Leiendecker 2013), and are efficient on seizures control but less on movement disorders (Leen et al. 2010). Triheptanoin, an odd-chain triglyceride with anaplerotic properties (i.e., providing both acetyl-CoA and propionyl-CoA that are key intermediates for the Krebs cycle), has recently opened new therapeutic perspectives for patients with Glut1-DS

Table 2 Metabolic forms of cerebellar ataxia amenable to treatments, with main clinical features and diagnostic biomarkers

Disease MIM number Inheritance	Gene	Main clinical features	Biomarkers	Treatments
Energy defects				
CoQ10 deficiency 612016 – AR	*ADCK3 (COQ8A)*	Cerebellar ataxia; exercise intolerance; mild–severe intellectual disability; seizures	CoQ10 ↓ in muscle biopsies	CoQ10
Glut1-DS 606777 – AD 612126 – AD 601042 – AD	*SLC2A1*	Intellectual disability; seizures; cerebellar ataxia; dystonia; paroxysmal movement disorders	CSF glucose ↓ METAglut1™ ↓	Ketogenic diets Triheptanoin
PDC deficiency 312170 – X-linked	*PDHA1*	Intellectual disability; hypotonia; seizures; cerebellar ataxia; peripheral neuropathy; dystonia	Lactate ↑ Pyruvate ↑; Lactate/pyruvate ratio N	Ketogenic diets Thiamine
Complex molecules accumulation				
Cerebrotendinous xanthomatosis 213700 – AR	*CYP27A1*	Chronic diarrhea; cataract; cognitive deficits; psychiatric features; cerebellar ataxia; spasticity; peripheral neuropathy	Plasma cholestanol ↑ 27-hydroxycholesterol ↓	Chenodeoxycholic acid
Niemann-Pick type C 257220 – AR 607625 – AR	*NPC1* (95%) *NPC2* (5%)	Juvenile-onset ataxia; vertical saccadic slowing; cognitive decline; dystonia; psychosis; hepatosplenomegaly	Oxysterols ↑ (cholestane-3b,5a,6b-triol; 7-ketocholesterol); Sphingolipids ↑ (lysosphingomyelin-509) Bile acids↑ (3b,5a,6b-trihydroxy-cholanoyl-glycine) Filipin test	Miglustat *HPβCD*? Arimoclomol?
Gaucher disease type 3 231000 – AR	*GBA1*	Progressive myoclonic epilepsy; seizures; oculomotor apraxia; ataxia	GBA1 activity ↓ Lysoglucosylceramide ↑	Ambroxol?
Metachromatic leukodystrophy 250100 – AR	*ARSA*	Variable depending on age at onset (late-infantile, juvenile, adult); affects both the central and peripheral nervous systems; ataxia mostly in juvenile and adult forms	ARSA activity ↓; Sulfatide urine ↑	HSCT

Krabbe disease 245200 – AR	*GALC*	Variable depending on age at onset (late-infantile, juvenile, adult); affects both the central and peripheral nervous systems; ataxia is most common in adult-onset forms	Galactocerebrosidase activity ↓	HSCT
Adrenoleukodystrophy 300100 – X-linked	*ABCD1*	Variable depending on age at onset; in adults, affects both the central and peripheral nervous systems; ataxia is most common in adult-onset forms	Very long-chain fatty acids ↑ C26:0-lysophosphatidyl-choline↑	HSCT Leriglitazone?
Refsum Disease 266500 – AR 614879 – AR	*PHYH* *PEX7*	Cerebellar ataxia; pigmentary retinopathy; sensory motor neuropathy; anosmia; hearing loss and ichthyosis	Phytanic acid ↑	Low-phytanic diet Plasma apheresis
Complex molecules deficiency				
SCA38 615957 – AD	*ELOVL5*	Spino-cerebellar ataxia; pes cavus; hyposmia	Arachidonic acid ↓ DHA ↓	DHA
Small molecules deficiency				
AVED 277460 – AR	*TTPA*	Cerebellar ataxia; sensory neuropathy; retinopathy	Vitamin E ↓	Vitamin E
Abetalipoproteinemia 200100 – AR	*MTTP*	Failure to thrive; hepatomegaly; cerebellar ataxia; sensory neuropathy; pigmentary retinopathy	Vitamin E ↓ Vitamins A,D,K ↓ Cholesterol ↓ Triglycerides ↓	Vitamin E + Vitamins A,D,K
Biotinidase deficiency 253260 – AR	*BTD*	Developmental delay; hearing loss; optic atrophy; skin rash; alopecia; cerebellar ataxia; spasticity	Urine β-hydroxyisovalerate ↑ Biotinidase activity ↓	Biotin
BBGD 607483 – AR	*SLC19A3*	Subacute encephalopathy with seizures; dystonia	None	Thiamine + Biotin

(continued)

Table 2 (continued)

Disease MIM number Inheritance	Gene	Main clinical features	Biomarkers	Treatments
BBVL2 614707 – AR	*SLC52A2*	Variable, including a form with hearing loss; optic atrophy; peripheral neuropathy; cerebellar ataxia	Short- and medium-chain (sometimes long-chain) acylcarnitines ↑	Riboflavin
Small molecules accumulation				
Urea cycle disorders 311250 – X-linked 215700 – AR 238970 – AR	*OTC* *ASS1* *SLC25A15*	Acute episodes of confusion; vomiting; ataxia	Ammonium ↑ Plasma amino acids chromatography (various profiles depending on deficit)	Low-protein diet

Abbreviations: *AD* autosomal dominant, *AR* autosomal recessive, *ARSA* arylsulfatase A, *AVED* ataxia with isolated vitamin E deficiency, *BBGD* biotin-thiamine-responsive basal ganglia disease, *BVVL2* Brown-Vialetto-Van Laere syndrome 2, *DHA* docosahexaenoic acid, *Glut1-DS* glucose transporter type 1 deficiency syndrome, *HPβCD* 2-hydroxypropyl-β-cyclodextrin, *HSCT* hematopoietic stem cell transplantation, *PDC* pyruvate dehydrogenase complex, *SCA38* spinocerebellar ataxia 38

with a great improvement in movement disorders (Mochel et al. 2016; Hainque et al. 2019)—triheptanoin is currently approved for the treatment of long-chain fatty acid oxidation defects in the USA. *Pyruvate dehydrogenase complex (PDC) deficiency* is a mitochondrial disorder of carbohydrate oxidation that mostly affects the brain and leads to decreased ATP production and energy deficit. PDH deficiency most commonly manifests as a syndrome of neurologic signs (congenital microcephaly, hypotonia, epilepsy, and/or ataxia), abnormal brain imaging (dysgenesis of the corpus callosum, Leigh syndrome), and metabolic abnormalities (increased plasma pyruvate, lactic acidemia, and/or metabolic acidosis). Rarely, individuals present later in childhood with intermittent ataxia (DeBrosse et al. 2012). Ketogenic diet has been shown to be particularly beneficial in decreasing seizures and improving ataxia, lactic acidosis, and sleep habits (Sofou et al. 2017). However, patients, especially adolescents and adults, have difficulties complying with the difficult constraints of ketogenic diets and, sometimes, side effects (e.g., growth retardation, steatosis). Other dietary interventions mainly comprise low-protein diets in the few patients with *urea cycle disorders* for whom hyperammonemia may be associated with acute episodes of cerebellar ataxia, as documented in ornithine transcarbamylase deficiency (Keller et al. 1998), citrullinemia (Saini et al. 2018), and hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (Ersoy Tunali et al. 2014).

3.2 Supplementation Therapies

Vitamins are the perfect examples of therapies that can easily correct a metabolic defect, hence, the importance of always considering cerebellar ataxias related to vitamin deficiencies. *Ataxia with isolated vitamin E deficiency* (AVED) is caused by pathogenic variants in the tocopherol-α transfer protein gene (TTPA). When mutated, *TTPA* prevents vitamin E to pass into general circulation. Patients manifest with a Friedreich ataxia-like phenotype, i.e., combined cerebellar and proprioceptive ataxia, Babinski sign, and a peripheral neuropathy associated with markedly decreased plasma vitamin E levels. High-dose oral tocopherol-α can stop or even reverse disease progression, with regression of ataxia (Gabsi et al. 2001). Initiation of treatment in the presymptomatic phase can prevent the development of symptoms (El Euch-Fayache et al. 2014). Similarly, *abetalipoproteinemia* is due to altered assembly and secretion of apolipoprotein B-containing lipoprotein particles due to pathogenic variants in *MTP* encoding the microsomal triglyceride transfer protein. This results in hypocholesterolemia and malabsorption of lipid-soluble vitamins, i.e., vitamins A, D, E, and K, leading to retinal degeneration, cerebellar ataxia, and sensory neuropathy. Treatment consists of a low-fat diet with oral supplementation of high-dose tocopherol-α, high-dose β-carotene, 25-hydroxy-vitamin D3, and vitamin K. As for AVED, the earlier the treatment is initiated, the better the outcome, with the exception of retinal function (Lee and Hegele 2014).

Vitamins B are another important group of vitamins to consider in patients with cerebellar ataxia, especially thiamine (B1), riboflavin (B2), and biotin (B8). Thiamine is routinely used in individuals with PDC deficiency, at doses of 300–1000 mg/day divided in three doses, although only a minority of individuals have shown biochemical or neurologic response—especially missense variants in exon 3 or the thiamine pyrophosphate binding site of the E1-α subunit (PDHA1)—being the most amenable to treatment (Sedel et al. 2008). Biotin is required for multiple biotin-dependent metabolic processes. *Biotinidase* recycles free, non-protein-bound biotin. Its deficiency can present as cerebellar ataxia in children or adults; other common symptoms usually comprise developmental delay, seizures, optic atrophy, deafness, and eczematous skin rash (Rahman et al. 1997). Motor and cutaneous symptoms respond very well to long-term biotin supplementation, even in adult-onset forms of the disease (Bottin et al. 2015). *Biotin-thiamine-responsive basal ganglia disease* is another inborn error of metabolism treatable with biotin, but also thiamine. It is caused by pathogenic variants in *SLC19A3*, which encodes human thiamine transporter 2. Because biotin is not a substrate for this transporter, the mechanism by which biotin is effective remains elusive. Patients usually manifest with subacute encephalopathy associated with cerebellar ataxia and dystonia (Debs et al. 2010). Brain imaging shows abnormal signal intensity with swelling in the basal ganglia during acute crises followed by atrophy of the basal ganglia. High oral dose of biotin, associated with thiamine, allows symptoms to resolve within days and usually prevents the reoccurrence of metabolic crises (Alfadhel et al. 2013). Recently, another form of cerebellar ataxia associated with optic atrophy, sensorineural deafness, and peripheral neuropathy has been linked to *SLC52A2 pathogenic variants* (Guissart et al. 2016). *SLC52A2* encodes a riboflavin transporter involved in Brown-Vialetto-Van Laere syndrome, which is responsive to riboflavin supplementation.

Besides vitamins, supplementation can be proposed to patients with cerebellar ataxia caused by a deficiency of cofactors, like coenzyme Q10, or downstream metabolites, like fatty acids. In cerebellar ataxia due to *ADCK3 pathogenic variants*, the synthesis of coenzyme Q10 itself is deficient. Supplementation by coenzyme Q10 seems to improve cerebellar ataxia in about half of *ADCK3*-mutated patients (Mignot et al. 2013; Traschütz et al. 2020). *Spinocerebellar ataxia 38* (SCA38) is caused by pathogenic variants in the *ELOVL5* gene, which encodes an elongase involved in the synthesis of polyunsaturated fatty acids, including docosahexaenoic acid (DHA). As a consequence, DHA is significantly reduced in the serum of SCA38 subjects (Manes et al. 2017). A double-blind randomized placebo-controlled study was conducted on ten patients with SCA38, which showed after 16 weeks a significant improvement in ataxia in the DHA group (Manes et al. 2017), confirmed during the 2-year open-label extension study (Manes et al. 2019).

3.3 Metabolite-Lowering Therapies

Cerebrotendinous xanthomatosis (CTX) is caused by pathogenic variants in the *CYP27A1* gene encoding the mitochondrial cytochrome P-450 enzyme sterol 27-hydroxylase. This defect interferes with the initial side chain oxidation step of the sterol intermediates in the alternative bile acid pathway. This results in reduced synthesis of chenodeoxycholic acid (CDCA) and shunt of sterol intermediates into the microsomal pathway for cholic acid formation (Salen et al. 1991). CTX is also associated with high production of cholestanol, which accumulates in different tissues, and increased levels of bile alcohols in urine (Berginer et al. 1984). Evidence that cholestanol may be neurotoxic is supported by the finding of cholestanol deposition and apoptosis in neuronal cells, most notably Purkinje cells, in the cerebellum of rats fed a 1% cholestanol diet (Inoue et al. 1999). Chronic diarrhea is often the first manifestations of the disease. Cataract and school difficulties may occur between 5 and 15 years of age preceding by years the onset of motor or psychiatric symptoms (Degos et al. 2016; Wong et al. 2018). Motor signs are dominated by cerebellar and/or pyramidal syndrome but a peripheral neuropathy is often associated. Cognitive decline and parkinsonism may further aggravate the disease course. CTX is easily diagnosed by measuring plasma cholestanol, followed by molecular analyses (Nie et al. 2014). CDCA remains the treatment of choice of CTX as it downregulates CYP7A, restores the imbalance between CDCA and cholic acid, and is the only drug that has shown effectiveness on neurological symptoms so far (Nie et al. 2014; Salen and Steiner 2017). The exogenous supply of CDCA acts by restoring a negative feedback in the endogenous acid bile and cholestanol synthesis. This drastically lowered plasma cholestanol concentrations in patients and its accumulation in tissues (Berginer et al. 1984; Nie et al. 2014). Long-term clinical benefit was demonstrated in most patients with CTX (Berginer et al. 1984; Mignarri et al. 2011; Yahalom et al. 2013), providing that treatment was initiated less than 15 years after the onset of neurological symptoms (Amador et al. 2018).

Refsum disease is caused by impaired α-oxidation of phytanic acid, either due to a deficiency in phytanoyl-CoA hydroxylase (*PHYH*) or *PEX7* pathogenic variants that affect the import of PHYH into the peroxisome. The resulting accumulation of the branched-chain fatty acid phytanic acid is thought to induce oxidative damage and mitochondrial dysfunction. PHYH knockout mice fed a diet supplemented with phytol, the precursor of phytanic acid, exhibited loss of Purkinje cells, possible through mitochondrial dysfunction (Busanello et al. 2013), and peripheral neuropathy (Ferdinandusse et al. 2008). Besides cerebellar ataxia, patients with Refsum disease present with variable degree of pigmentary retinopathy, sensory motor neuropathy, anosmia, hearing loss, and ichthyosis. Screening for the disease can be performed by measuring increased plasma phytanic acid levels. A significant lowering of phytanic acid concentrations can be obtained in Refsum disease by reducing dietary intake of phytanic acid while ensuring sufficient calorie intake to avoid mobilization of stored lipids, including phytanic acid, into the plasma. Long-term benefit of dietary intervention was shown on phytanic acid levels (Baldwin et al. 2010). However, this may be not sufficient in some patients to prevent acute attacks

and stabilize the progressive course. Lipid apheresis can then be a complementary or alternative therapeutic strategy to lower phytanic acid concentrations (Zolotov et al. 2012). Reduction in plasma phytanic acid concentration is usually accompanied by improved ichthyosis, sensory neuropathy, and ataxia.

Niemann-Pick type C (NPC) disease affects primarily the trafficking of intracellular cholesterol in the brain and peripheral organs. The disease is due to pathogenic variants in the *NPC1* (95%) and *NPC2* (5%) genes that encode respectively a large membrane protein and a small soluble protein. Both proteins localize at the late endosomal/lysosomal compartment (Vanier et al. 1996). NPC1/2 deficiency results in altered processing and utilization of endocytosed cholesterol, which leads to the storage of unesterified cholesterol and secondary alterations of sphingolipid metabolism, especially increased GM2 and GM3 gangliosides in cerebral gray matter (Vanier 2015). Sphingolipid accumulation appears secondary to lysosomal cholesterol storage but the mechanisms underlying such alterations remain elusive. Neuropathological features comprise neuronal storage with meganeurite formation and extensive growth of ectopic dendrites, possibly linked to abnormal ganglioside accumulation, and formation of neurofibrillary tangles, possibly linked to abnormal cholesterol trafficking (Walkley and Suzuki 2004). As the disease progresses, neuronal death affects predominantly Purkinje cells of the cerebellum. NPC may manifest from the perinatal period to late adulthood. Liver disease is observed during infancy or childhood in about one-third of patients. Neurological symptoms may develop in childhood, adolescence, or adulthood, especially cerebellar ataxia, cognitive decline, movement disorders, and seizures. NPC has been shown to cause early-onset ataxia (Schicks et al. 2013). Nonetheless, psychiatric symptoms, from psychosis to depression, may precede by years the occurrence of motor symptoms (Sevin et al. 2007). Vertical supranuclear saccade palsy is one of the first and most frequent signs in patients with NPC. Plasma biomarkers are now considered first line diagnostic tools, especially oxysterols (cholestane-3b,5a,6b-triol being more specific and sensitive than 7-ketocholesterol), sphingolipids (lysosphingomyelin-509), and bile acids (3b,5a,6b-trihydroxy-cholanoyl-glycine) (Patterson et al. 2017). However, none are specific for NPC as they can also detect patients with Niemann-Pick type A and B. The diagnosis of NPC needs to be confirmed by molecular analyses of *NPC1* and *NPC2*. The filipin staining test, which demonstrates impaired trafficking of endocytosed cholesterol in living fibroblasts, remains critical for patients for whom genetic testing has not allowed the identification of two pathogenic variants (Patterson et al. 2017). Therapeutic strategies for NPC disease have included pharmacologic inhibition of substrate accumulation. Miglustat is a substrate reduction therapy that aims at lowering glycolipid storage in NPC. It inhibits glucosylceramide synthase and stabilizes disease progression in adolescent and adult patients (Patterson et al. 2007; Wraith et al. 2010) but is not yet approved by the US Food and Drug Administration (FDA). Frequent adverse events are diarrhea and weight loss. Cyclodextrins are thought to replace the function of NPC1/2 within the late endosome/lysosome compartment. Administration in mouse and cat models of NPC reduces unesterified cholesterol and glycolipid accumulation within the brain and peripheral organs (Aqul et al. 2011). In an open-label trial, 14 patients

with NPC received intrathecal increasing doses of 2-hydroxypropyl-β-cyclodextrin (HPβCD) and showed slower disease progression compared to a historical cohort of 21 patients (Ory et al. 2017). In another case series, moderately affected NPC patients treated with HPβCD showed slowing of disease progression (Hastings et al. 2019). A phase 2/3 trial is ongoing (NCT02534844). However, 2-hydroxypropyl-b-cyclodextrin is ototoxic, so safer alternate cyclodextrins should be considered (Davidson et al. 2016). Arimoclomol, an orally available small molecule that crosses the blood-brain barrier and activates the heat shock protein 70 (HSP70), can improve the binding of several sphingolipid-degrading enzymes and attenuated a wide spectrum of disease-associated neurological symptoms in Npc1(−/−) mice (Kirkegaard et al. 2016). In a 12-month, randomized, double-blind, placebo-controlled, phase 2/3 trial, where patients (2–18 years) were randomized 2:1 to arimoclomol placebo, stratified by miglustat use, a significant change in the 5-domain NPC Clinical Severity Scale (NPCCSS) score was observed from baseline to 12 months, corresponding to a 65% reduction in annual disease progression (Mengel et al. 2021).

Gaucher disease is caused by GBA1 deficiency that leads to the buildup of glucosylceramide. The most common neurological phenotype (type 3) encompasses progressive myoclonic epilepsy, seizures, and oculomotor apraxia. Ataxia has been mainly observed in the later stages of the disease (Winkelman et al. 1983). Miglustat was also used in neuronopathic Gaucher disease to decrease glucosylceramide concentrations but failed to show efficacy (Schiffmann et al. 2008). Novel substrate reduction therapies are being developed with more targeted actions on the central nervous system and therefore fewer side effects (Peterschmitt et al. 2021).

3.4 Chaperone and Replacement Therapies

For cerebellar ataxias related to enzymatic deficiencies, drugs have been developed that can increase residual enzymatic activity while reaching the central nervous system, unlike most systemic enzyme replacement therapies due to the large size of enzymes. Likewise, pharmacological chaperones are small molecules that correct the folding of misfolded proteins, allowing them to pass through the cell's quality-control system. Most of them are still under development in IMD causing cerebellar ataxia. Nonetheless, ambroxol, a commercially available expectorant, was shown to enhance glucocerebrosidase activity in mouse brain (Maegawa et al. 2009). A pilot open-label study was conducted in five patients with neuronopathic *Gaucher disease*: high-dose oral ambroxol increased glucocerebrosidase activity, decreased cerebrospinal fluid (CSF) glucosylceramide concentrations, and showed some clinical benefit (Narita et al. 2016). Other case studies have reported a possible clinical stabilization when ambroxol was used in combination with enzyme replacement therapy (Charkhand et al. 2019).

Galactosylceramide and sulfatides are major glycosphingolipids in the myelin sheath. Arylsulfatase A (ARSA) initiates the degradation of sulfatides and produces

galactosylceramide. ARSA deficiency causes various subtypes of *metachromatic leukodystrophy* (MLD) characterized by sulfatide accumulation and decreased galactosylceramide, both contributing to the myelin pathology (Sevin et al. 2007). Excess in sulfatides may also trigger microglial activation (Jeon et al. 2008). Unlike patients, ARSA-deficient mice display significant loss of Purkinje cells but no cerebral demyelination (Matzner et al. 2005). Cerebellar ataxia is mostly observed in the juvenile and adult forms of MLD for which genotype–phenotype correlations have been reported (Rauschka et al. 2006). Sulfatiduria is a biomarker of MLD. *Krabbe disease* is caused by galactocerebrosidase deficiency, a lysosomal enzyme that catabolizes the hydrolysis of galactose from galactosylceramide and psychosine. Unlike MLD, patients with Krabbe disease present increased concentrations of galactosylceramide, which is toxic for oligodendrocytes differentiation and survival (Won et al. 2013). Rare late-onset forms of Krabbe disease have also been observed with predominant cerebellar ataxia (Shao et al. 2016). Replacement therapies can be achieved by intrathecal enzyme replacement therapy or cell therapy such as hematopoietic stem cell transplantation or gene therapy. In MLD, a phase 1/2 dose-escalation study designed to evaluate the safety of intrathecal enzyme replacement therapy showed that CSF sulfatide and lysosulfatide levels fell to within normal ranges at a dose of 100 mg of recombinant human ARSA every other week (Dali et al. 2020). Although there was a general decline in motor function over time, there was a tendency toward a less pronounced decline in patients receiving 100 mg (Dali et al. 2020). Hematopoietic cells, including activated lymphocytes, monocytes, and microglia precursors, hold the advantage to cross the blood-brain barrier after systemic administration. In a post-mortem study, it was shown that donor macrophages are able to digest accumulated sulfatides and may play a neuroprotective role for resident oligodendrocytes, thereby enabling remyelination, albeit without evidence of enzymatic cross-correction of oligo- and astroglia (Wolf et al. 2020). These results emphasize the importance of immunomodulation in addition to the metabolic correction, which might be exploited for improved outcomes. Hematopoietic stem cell transplantation can stabilize cerebral demyelination in patients with juvenile and adult-onset forms of MLD (Groeschel et al. 2016; van Rappard et al. 2016) but seems to have no impact on the development of cerebellar ataxia and peripheral neuropathy. A non-randomized phase 1/2 trial using hematopoietic stem cell gene therapy in presymptomatic and very early symptomatic stage of MLD resulted in sustained, clinically relevant benefits in children with early-onset MLD by preserving cognitive function and motor development in most patients, and slowing demyelination and brain atrophy (Fumagalli et al. 2022). Among patients with late-onset forms of Krabbe disease, hematopoietic stem cell transplantation may also be beneficial (Lim et al. 2008; Laule et al. 2018). Cerebellar ataxia can also be the presenting symptom of adult-onset forms of *cerebral adrenoleukodystrophy* (CALD) (Chen et al. 2017). While hematopoietic stem cell transplantation performed at the early stage of CALD can arrest disease progression thanks to its neuroimmune actions (Kühl et al. 2017), cerebellar involvement is considered of poor prognosis (Waldhüter et al. 2019). Recently, leriglitazone, a novel selective peroxisome proliferator-activated receptor gamma agonist, was shown to reduce the occurrence of CALD (Köhler et al. 2023). Further

studies will investigate whether leriglitazone can reduce disease progression in patients with CALD, especially those who are ineligible to transplant.

4 Conclusion

In case of chronic cerebellar ataxia, a fast and simple metabolic screening can identify etiologies that are amenable to treatment, especially vitamin E, CSF glucose—or METAglut1™ if available—plasma cholestanol, very long-chain fatty acids, phytanic acid, and lysosphingomyelin-509. If magnetic resonance imaging (MRI) reveals an abnormal white matter, then measuring the activities of arylsulfatase A and galactocerebrosidase is warranted. In case of paroxysmal episodes of cerebellar ataxia, CSF glucose—or METAglut1™ if available—plasma lactate, pyruvate, ammonium, and amino acid chromatography are of utmost importance. The most common metabolic causes of cerebellar ataxia that best respond to treatments are Glut1-DS, cerebrotendinous xanthomatosis, AVED, and abetaliproteinemia, as well as Refsum disease.

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Clinical Trials in Fragile X-Associated Tremor/Ataxia Syndrome

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Abstract Fragile X-associated tremor/ataxia syndrome (FXTAS) is a rare, adult-onset neurodegenerative movement disorder occurring in some carriers of a 55–200 CGG repeat "premutation" in the fragile X mental retardation 1 (*FMR1*) gene that is characterized by cerebellar gait ataxia, intention tremor, and executive dysfunction. The disease is progressive and can lead to significant disability, increased fall risk, and reduced lifespan. There are still no proven symptomatic or neuroprotective treatments for FXTAS, and the development of clinical trials has been limited by a lack of valid and reliable outcome measures. Preclinical work in *Drosophila* and mouse models of FXTAS has provided the rationale behind three clinical drug trials that have been completed in FXTAS to date. In this monograph, we review the three Phase II clinical trials of memantine, allopregnanolone, and citicoline, and one exercise intervention trial that has been completed to date, and the implications of findings from these studies. Memantine, a cognitive enhancer used to treat Alzheimer's disease, demonstrated no treatment effects after 12 months of study; however, a sub-analysis indicated that the drug may enhance attentional processes. The 12-week trial of allopregnanolone, an endogenous neurosteroid, demonstrated significant improvement in executive function and episodic learning and memory in a subset of patients; however, no significant changes were seen in the characteristic neurological motor signs of FXTAS. In the trial of citicoline, a phospholipase A_2 inhibitor, no significant change in symptoms was seen over the course of the 12-month study; however, decline would have been expected, suggesting that the compound may have helped to stabilize disease progression. The 3-month treadmill-training pilot study was also demonstrated to be a feasible and safe intervention. The favorable outcomes and safety profiles of the three clinical trials support the conduct of Phase III studies, and preliminary results of the treadmill-training study

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_26

are also promising and highlight the importance of developing non-pharmaceutical therapies for FXTAS.

Keywords Clinical trials · FXTAS · Memantine · Allopregnanolone · Citicoline · Treadmill training

1 Introduction

First described in 2001, fragile X-associated tremor/ataxia syndrome (FXTAS) is a rare, adult-onset neurodegenerative movement disorder caused by repeat-associated non-ATG (RAN) translation and a toxic RNA gain of function occurring in some carriers of a 55–200 CGG repeat "premutation" in the *fragile X mental retardation 1 (FMR1)* gene, as well as some individuals with gray zone alleles containing 45–54 CGG repeats (Hagerman et al. 2001; Todd et al. 2013). The pathophysiology of FXTAS is characterized by neuronal and glial cell intranuclear inclusions containing neurotoxic mRNA and FMRpolyG protein, which cause neurodegeneration and lead to the FXTAS phenotype (Sellier et al. 2014). Additional features include atrophy of the cerebellum, with extensive Purkinje cell loss, as well as progressive global brain atrophy and white matter disease (Greco et al. 2006). White matter lesions are typically seen in the brainstem or middle cerebellar peduncles, a phenomenon known as the "MCP" sign (Hall et al. 2016). Core motor and cognitive features include intention tremor, cerebellar gait ataxia, and executive dysfunction, which are progressive and can lead to significant disability, increased fall risk, and reduced lifespan. There is high phenotypic variability, with some carriers also exhibiting dementia, neuropsychiatric problems, parkinsonism, autonomic dysfunction, and peripheral neuropathy (Jacquemont et al. 2003). FXTAS is considered to be a rare disease, estimated to affect 30% of *FMR1* premutation carrier men over the age of 55, or ~31,000 men in the United States (Hagerman et al. 2008; Tassone and Hagerman 2012). The *FMR1* gene is located on the X chromosome; therefore, penetrance and severity of FXTAS is lower in women due to the presence of a second normal protective *FMR1* allele and the phenomenon of X-inactivation, in which the *FMR1* premutation is only active in half of cells on average. Of the approximately 785,000 premutation carrier women in the United States, it is estimated that 15% will manifest symptoms in their lifetime, which can include primary ovarian insufficiency occurring in up to 20% of premutation women (Hall et al. 2016).

One of the hallmark motor features of FXTAS is the cerebellar gait ataxia and degenerative loss of motor coordination seen in approximately 41–66% of patients (Juncos et al. 2011; Niu et al. 2014). Previous studies have characterized the gait and balance deficits in FXTAS patients and found them to be similar to those seen in other cerebellar disorders (O'Keefe et al. 2015; Trouillas et al. 1997). Tremor, another hallmark feature, has been observed in approximately 77% of men with FXTAS (Juncos et al. 2011) and typically consists of bilateral postural or kinetic tremor of the upper extremities, with rest tremor being uncommon. A subset of

FXTAS patients (29–32%) also present with a mild form of parkinsonism that may be associated with having a smaller *FMR1* expansion or a gray zone allele (Hall et al. 2012; Robertson et al. 2016).

The primary cognitive phenotype in FXTAS includes executive dysfunction that may later develop into global dementia in as many as 50% of men with FXTAS and a smaller but unknown number of FXTAS women. Deficits in processing speed, verbal fluency, response inhibition, attentional control, and working memory have all been described in FXTAS men (Grigsby et al. 2008). Abnormalities in these domains have also been seen in some FXTAS women, although they have not been studied as extensively as men. FXTAS men and women exhibit elevated levels of mood and anxiety disorders compared to the general population (Bourgeois et al. 2009), with psychosis rarely seen.

2 Clinical Trials in FXTAS

Preclinical work in *Drosophila* and mouse models of FXTAS has provided the rationale behind three Phase II clinical trials and one exercise intervention trial that have been completed in FXTAS to date and have demonstrated the feasibility of recruiting and retaining FXTAS patients for clinical trials. Here we will review the trials of memantine, allopregnanolone, and citicoline (Table 1). A fourth preliminary trial using a treadmill-training paradigm will also be discussed.

3 Memantine

The first, and only to date, double-blind randomized clinical trial conducted in FXTAS investigated the cognitive enhancer memantine, an uncompetitive *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist approved by the Food and Drug Administration for the treatment of Alzheimer's disease (AD) (Seritan et al. 2014). Memantine was chosen for the trial based on preclinical work in an *FMR1* premutation mouse model, which identified abnormal glutamate activity in hippocampal neurons (Cao et al. 2012). NMDA antagonists exert a neuroprotective effect through decreasing NMDA receptor calcium ion channel permeability leading to a reduction in apoptosis (Reisberg et al. 2003), as well as mitigating impairments in long-term potentiation (Zajaczkowski et al. 1997); therefore, it was hypothesized that memantine would ameliorate motor, cognitive, and behavioral symptoms in FXTAS patients. Memantine has previously been used in clinical trials to improve neurological, cognitive, and behavioral symptoms in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease (PD), Lewy body dementia, and vascular dementia, as well as in psychiatric disorders and Down's syndrome, with mixed results (Seritan et al. 2014). Additionally, a case report showed neurological and cognitive symptom improvement in one female premutation carrier with administration of memantine combined with venlafaxine (Ortigas et al. 2010).

Table 1 Clinical drug trials in FXTAS

Study and year published	Author	Compound characteristics	Mechanism of action	Previous use in other diseases	Preclinical animal model	Treatment frequency and duration	Study population	Primary outcome measures	Results
Memantine, 2014	Seritan et al.	Non-competitive NMDA glutamate receptor antagonist	Decrease NMDA receptor calcium ion channel permeability leading to a reduction in apoptosis	AD; PD; LBD; vascular dementia; TBI; psychiatric disorders; Down's syndrome	*Mouse*	Daily for 12 months	60 men, 34 women with FXTAS	Behavioral Dyscontrol Scale; CATSYS intention tremor assessment	Negative for treatment effects for primary or secondary outcomes
Memantine sub-study, 2016	Yang et al.	Non-competitive NMDA glutamate receptor antagonist	Decrease NMDA receptor calcium ion channel permeability leading to a reduction in apoptosis	AD; PD; LBD; vascular dementia; psychiatric disorders; neuropathic pain; Down's syndrome	*Mouse*	Daily for 12 months	48 subjects from original memantine study	ERP parameters during auditory "oddball" task	Significant improvement in N400, late positive component, P2 amplitude

Allopregnanolone, 2017	Wang et al.	Endogenous neurosteroid	Positive modulation of $GABA_A$ receptors	Epilepsy	*Mouse*	Weekly for 3 months	6 men with FXTAS	Working memory; executive function; learning and memory; CATSYS tremor and sway assessments	Negative for treatment effects; significant correlation between baseline MRI and improvement in executive and psychological function
Citicoline, 2020	Hall et al.	Endogenous nucleotide intermediate in the biosynthesis of structural membrane phospholipids	Phospholipase A_2 inhibitor	Brain aging; head trauma; stroke; cerebrovascular pathology; AD; mild dementia	*Drosophila*	Daily for 12 months	9 men, 1 woman with FXTAS	FXTAS-RS	No change in FXTAS-RS; reduced anxiety; improved response inhibition

Abbreviations: *AD* Alzheimer's disease, *CATSYS*, Coordination Ability and Tremor System, *ERP* event-related potential, *FXTAS* fragile x-associated tremor/ataxia syndrome, *FXTAS-RS* FXTAS Rating Scale, *$GABA_A$* gamma-aminobutyric $acid_A$, *LBD* Lewy body dementia, *MRI* magnetic resonance imaging, *NMDA* *N*-methyl-D-aspartate, *PD* Parkinson's disease, *TBI* traumatic brain injury

The trial included 60 men and 34 women with FXTAS treated with 10 mg of memantine twice per day for 12 months (Seritan et al. 2014). Primary outcome measures included two measures that have been widely used in premutation carrier studies: the Behavioral Dyscontrol Scale, a measure of executive function (Grigsby et al. 2008), and a quantitative measure of intention tremor obtained by the Coordination Ability and Tremor System (CATSYS), which is a machine-based assessment tool that has been used in previous studies in premutation carriers and shown to be associated with clinician-rated Unified Parkinson's Disease Rating Scale items (Narcisa et al. 2011). Secondary outcome measures included CATSYS assessment of postural and writing tremor and hand and finger tapping, as well as additional cognitive tests of learning, memory, and executive function. No treatment effects were seen for primary or secondary outcome measures after 12 months of study.

A sub-study of the memantine trial investigated its effects on attention and working memory via cognitive event-related potential (ERP) measurement in a subset of 48 study patients from the original memantine trial who underwent electroencephalogram (EEG) recording during an auditory paradigm targeting attentional processes at baseline and 12-months (Yang et al. 2016). This auditory "oddball" paradigm has been used previously in FXTAS and revealed ERP abnormalities in men with FXTAS but not in asymptomatic carrier women (Yang et al. 2014a). In the sub-study, signs of significant improvement following memantine treatment were found in respect to the auditory ERP P2 amplitude, a component that is thought to reflect attentional processes (Fig. 1). This suggests that memantine may be beneficial for improving executive function, the core cognitive feature of FXTAS. It was noted that the cognitive effects of memantine may be attributed to its dopaminergic agonist properties and that it may be worthwhile to investigate the effects of memantine on psychiatric symptoms in FXTAS in future studies.

A second sub-study used a word repetition paradigm during EEG to analyze the N400 and P600 ERP components in a subset of 41 patients from the original memantine trial in order to investigate the effects of 12 months of memantine treatment on verbal memory (Yang et al. 2014b). Previous ERP studies in FXTAS have found a reduction in the N400 repetition effect (Olichney et al. 2010), which is an established marker of verbal memory processes. Results from this sub-study demonstrated benefits for cued-recall memory abilities and N400 repetition effects in the memantine group compared to the placebo group, with the proposed mechanism being regulation of glutamatergic signaling abnormalities (Yang et al. 2014b). However, this treatment effect was judged to be modest due to study limitations and did not extend to free recall or recognition memory. Nonetheless, this sub-study suggests that EEG/ERP measures may be sensitive to detect treatment effects in future clinical trials in FXTAS.

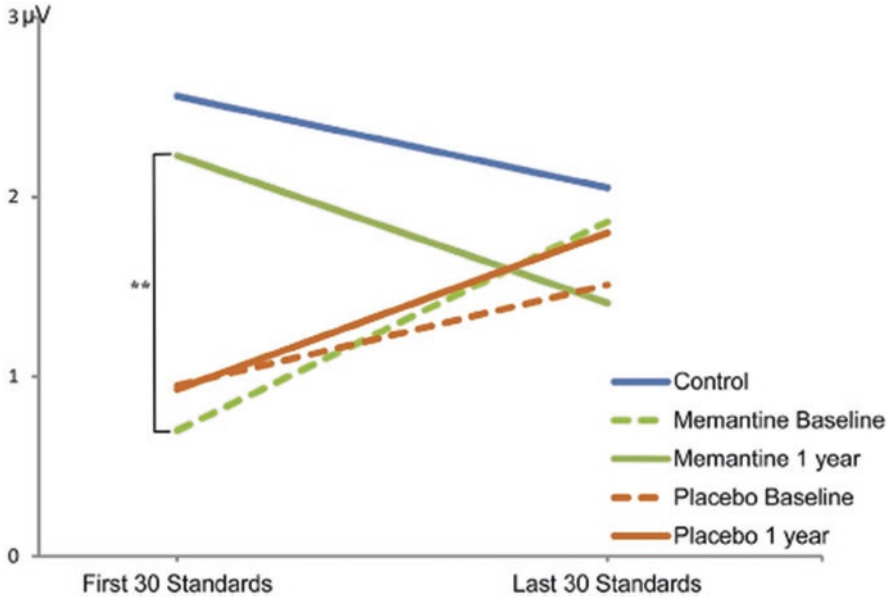

Fig. 1 P2 amplitudes (μV) to the first 30 standard tones and the last 30 standard tones within the first block of each EEG study, showing a reduced amplitude of responses to the later stimuli (i.e., a habituation effect) in the memantine-treated FXTAS group (solid green) and a normal control group (blue), but increased amplitudes to later stimuli in the other groups (Yang et al. 2016)

4 Allopregnanolone

Findings from the premutation mouse model again set the precedent for an open-label uncontrolled Phase II trial investigating allopregnanolone, an intravenous endogenous neurosteroid and positive modulator of gamma-aminobutyric $acid_A$ ($GABA_A$) receptors (Wang et al. 2017). In the mouse model, hippocampal neurons extracted from hemizygous male mice carrying 170 *FMR1* CGG repeats exhibited elevated *FMR1* mRNA and reduced FMRP levels along with deficiencies in GABA and glutamate transport and abnormal cluster burst firing patterns. These abnormalities were rescued upon exposure to allopregnanolone in a dose-dependent and reversible manner, supporting the conduct of a clinical trial of this compound in premutation carriers with FXTAS (Cao et al. 2012).

In the trial, allopregnanolone was administered weekly to 6 FXTAS men over 12 weeks. Outcome measures included cognitive measures of working memory, executive function, learning and memory, and neurological motor symptoms measured by CATSYS tremor and sway assessments. Mitochondrial function in lymphocytes and magnetic resonance imaging (MRI) scans of brain structures that are sometimes affected in FXTAS (hippocampus, amygdala, corpus callosum) were also assessed for changes in neurodegeneration and white matter integrity, and

N400 repetition effects were measured using EEG recording. Results of the trial demonstrated significant improvement in executive function and episodic learning and memory in a subset of patients, although it is possible that these improvements were due to a placebo effect. No significant changes were seen in the characteristic neurological motor signs of FXTAS (tremor/ataxia), MRI measurements, or mitochondrial function before and after treatment, although subtle improvements in MRI measurements were seen in a subset of patients supporting the conduct of future trials over a longer time period to assess for potential disease modification. Further analysis also revealed significant correlations between baseline MRI measurements and changes in executive function and psychological symptoms following allopregnanolone treatment, further indicating that controlled trials with larger subject samples are warranted.

5 Citicoline

Preclinical data from a chemical screen using a *Drosophila* model demonstrated that phospholipase A_2 inhibitors can specifically suppress locomotion deficits caused by fragile X premutation rCGG repeats and reduce lethality (Qurashi et al. 2012). These findings provided the rationale for a second open-label Phase II clinical trial investigating citicoline (CDP-choline), an endogenous nucleotide intermediate in the biosynthesis of structural membrane phospholipids and a phospholipase A_2 inhibitor that is available for over-the-counter use. Citicoline has been used to treat neurodegenerative diseases associated with brain aging, head trauma, stroke, and cerebrovascular pathology, and has been shown to improve cognitive performance in patients with Alzheimer's disease (AD) and mild dementia (Alvarez et al. 1999).

The aim of this study was to evaluate the safety and efficacy of citicoline over a 12-month period in nine men and one woman with FXTAS using a similar drug regimen to that used in studies conducted in stroke and AD. The primary outcome measure in the study was the FXTAS Rating Scale (FXTAS-RS) score, which is a scale that was developed shortly after the disease was discovered to measure the salient movement disorders seen in FXTAS using selected items from three published rating scales—the Clinical Rating Scale for Tremor for tremor assessment, the International Cooperative Ataxia Rating Scale for ataxia assessment, and the Unified Parkinson's Disease Rating Scale, part III, for parkinsonism assessment—and an item testing tandem gait from the Unified Huntington's Disease Rating Scale (Hall et al. 2019). A battery of neuropsychological tests was used to evaluate cognitive function including executive function, verbal fluency, processing speed, and working memory and attention. Patient-reported balance confidence, anxiety, and depression were measured using questionnaires. Motor testing included an instrumented Timed Up and Go test (i-TUG; APDM™; Oregon), computerized dynamic posturography (Neurocom™, Natus Medical Inc., 2009), and the 9-hole peg test (Kellor et al. 1971).

No significant change in FXTAS-RS score was seen over the course of the study; however, decline would have been expected in this population over a 12-month period, suggesting that citicoline may have helped to stabilize disease progression. Additionally, a significant reduction in anxiety scores and improvement in response inhibition was seen, although further study is needed to determine whether these were true effects rather than false positives. Regardless, secondary outcome measures remained stable overall over the course of 1 year. Several patients experienced adverse events during the course of the study but these were generally not attributed to the study drug, except for gastrointestinal issues in one patient and an episode of acute vertigo in another patient, which were deemed to be possibly related (Hall et al. 2020). Citicoline was also determined to be safe and well tolerated in FXTAS. Although research suggests that the placebo effect in FXTAS is minimal at best (Hill et al. 2020), a larger study with a placebo arm is warranted to more accurately determine the efficacy of citicoline.

6 Treadmill Training

Recently, a pilot trial was conducted to determine the feasibility and safety of a treadmill-training intervention for improving gait ataxia in FXTAS (O'Keefe JA, Bang D, Hall DA, 2021, Feasibility of dual-task treadmill training to improve gait and balance in patients with FXTAS: a pilot trial, "unpublished"). Cerebellar gait ataxia, along with cognitive decline, may be prognostic of falls, progressive disability, and reduced quality of life. Cardiovascular exercise training while performing a simultaneous cognitive task typically referred to as a "dual-task" intervention has been demonstrated to improve both motor and cognitive functions in patients with PD, traumatic brain injury, and chronic stroke (Kim et al. 2016; Yogev-Seligmann et al. 2012; Parrington et al. 2020). Therefore, it was hypothesized that a dual-task intervention targeting gait, balance, and cognitive deficits may have the potential to improve function and slow disease progression in FXTAS. The dual-task treadmill-training program was developed and administered three times per week to a FXTAS intervention group and compared to a no intervention FXTAS control group. The sessions consisted of 30–45 minutes of aerobic exercise at 65% of a patient's maximum heart rate while performing an executive function task (Fig. 2). Four subjects (three in the intervention group; one in the control group) completed the study from baseline though at least the three-month follow-up visit. The study was halted prior to the six-month follow-up visit due to the Coronavirus Disease-2019 (COVID-19) pandemic, as well as worsening symptoms in two control subjects with FXTAS. Preliminary results indicate that dual-task treadmill training is a feasible and safe intervention for FXTAS. Improvements in several secondary outcome measures including balance, several gait parameters, cardiopulmonary function, and anxiety were also seen and provide preliminary evidence that further investigation is warranted. This pilot study will inform the design and conduct of a larger future dual-task treadmill-training clinical trial in FXTAS.

Fig. 2 Subjects walked on the treadmill for 30 minutes while performing a working memory task with a motor target of 60–80% of maximum heart rate and a cognitive target of 90% correct answers (O'Keefe JA, Bang D, Hall DA, 2021, Feasibility of dual-task treadmill training to improve gait and balance in patients with FXTAS: a pilot trial, "unpublished")

7 Conclusions

Despite knowing the exact mutation responsible for the disease, there is currently no proven therapeutic treatment for FXTAS. However, the clinical trials that have been completed thus far in FXTAS populations have identified compounds that are well tolerated and could potentially improve or stabilize cognitive and motor disease symptoms and are worthy of further investigation. The memantine, allopregnanolone, and citicoline trials resulted in favorable outcomes and safety profiles, supporting the conduct of Phase III studies. Preliminary results of the dual-task treadmill-training study are also promising and highlight the importance of developing non-pharmaceutical therapies for FXTAS. However, clinical trial methodology in FXTAS is underdeveloped and not yet adequate for the conduct of multi-site clinical trials or Phase III studies. The development of FXTAS outcome measures that are salient, validated, and appropriate for use in Phase III clinical trials is

critical to the teams that have pioneered work in this area, continuing the momentum of basic scientists in the field who are still identifying therapeutic targets. The exploration of antisense oligonucleotides (ASOs) as therapeutic strategies is rapidly expanding in a variety of neurodegenerative disease fields, including FXTAS. Work is currently underway to determine the therapeutic potential of modified ASOs to directly target *FMR1* mRNA and reduce the RNA toxicity effects of the CGG repeat expansion on multiple levels, such as by decreasing FMRpolyG production (Derbis et al. 2021). Thus far, studies have shown that mutant *FMR1* can be targeted at multiple stages of gene expression, rescuing molecular and behavioral phenotypes in the mouse model, and positioning ASOs as a promising therapeutic target for treatment delivery in FXTAS and other CGG repeat and related disorders.

Although the disease was discovered relatively recently, rapid identification of affected individuals within fragile X families and a shift of fragile X researchers into FXTAS-related projects has advanced the field into early clinical trials. Research efforts have been aimed toward understanding the clinical and molecular features associated with the *FMR1* premutation and identifying small molecule drug candidates. Although the clinical trials reviewed here have demonstrated the feasibility of recruiting and retaining FXTAS patients for future trials, these studies have failed to move on to Phase III trials due, in part, to small sample sizes, absence of standardized clinical outcome assessments, and lack of patient-reported outcome measures. Successful Phase III clinical trials are desperately needed for translational scientists to move promising therapies from preclinical testing into the clinic, and the FXTAS research community is actively working on developing FXTAS-specific outcome measures that may be more accurate for measuring neurological signs in clinical trials.

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Part V
Sporadic Ataxias

Therapeutic Strategies in Immune-Mediated Cerebellar Ataxias

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Abstract Immune-mediated cerebellar ataxias (IMCAs) include diverse etiologies, suggesting that the cerebellum can be the target of many types of autoimmune responses with different pathophysiological mechanisms. When a known factor triggers autoimmunity, the first line of therapy is removal of the triggering factor, for example gluten-free diet in gluten ataxia, and surgical excision or chemotherapy of the neoplasm in paraneoplastic cerebellar degeneration (PCD). Certain conditions (e.g., post-infectious cerebellitis, Miller Fisher syndrome, and post-infectious opsoclonus myoclonus syndrome) are self-limiting presumably because exposure of antigen is transient. However, due to persistent stimulation by autoantigens (if the cancer cannot be eradicated), PCD requires subsequent combinations of immunotherapies that on the whole tend to be ineffective. For other types of IMCAs, such as anti-glutamic acid decarboxylase (anti-GAD) ataxia and primary autoimmune cerebellar ataxia (PACA), immediate immunotherapy is recommended. The cerebellum, a vulnerable autoimmune target of the nervous system, has remarkable capacity (collectively known as the *cerebellar reserve*, closely linked to plasticity) to compensate and restore function following various pathological insults. Therefore, a good recovery is expected when immune-mediated therapeutic interventions are applied during the early stages when the cerebellar reserve can be preserved, halting the progression and the development of significant disability. We recommend careful examination of the autoimmune profile in all patients presenting with progressive ataxia. This chapter does not discuss the topic of multiple sclerosis (MS).

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_27

Keywords Immune-mediated cerebellar ataxias · Immunotherapies · Cerebellar reserve

1 Introduction

Immune-mediated cerebellar ataxias (IMCAs) involve diverse etiologies, including post-infectious cerebellitis (PIC), Miller Fisher syndrome (MFS), gluten ataxia (GA), paraneoplastic cerebellar degeneration (PCD), opsoclonus myoclonus syndrome (OMS), and anti-glutamic acid decarboxylase (anti-GAD) ataxia (Hadjivassiliou 2012; Mitoma et al. 2015, 2016, 2021a; Joubert et al. 2018; Hadjivassiliou et al. 2019; Joubert and Honnorat 2019). When the clinical profile does not match any of these established etiologies, these conditions are categorized under the term of primary autoimmune cerebellar ataxia (PACA) (Hadjivassiliou et al. 2008a, 2020). From the autoimmune background, these etiologies can be classified into two categories: (1) autoimmunity is triggered by another condition, including infection (PIC, MFS, OMS), gluten sensitivity (GA), and neoplasm (PCD, OMS) (Mitoma et al. 2016, 2021a), and (2) autoimmunity is not apparently triggered by another condition. This includes PACA. From a pathophysiological point of view, the cerebellum can be affected also through diverse immune mechanisms. For example, cell-mediated autoimmunity underlies PCD, while autoantibody-mediated functional disorders are also involved in anti-GAD ataxia (Mitoma et al. 2021a). The presence of various types of etiology-specific autoantibodies could reflect various autoimmune mechanisms (Table 1).

These autoantibodies include pathogenic antibodies (Abs) and non-pathogenic and marker Abs. So far, only a few pathogenic Abs have been identified (Table 1). Abs toward cell-surface receptors and ion channel-related proteins are considered to be pathogenic, despite only a few experimental evidence (Mitoma et al. 2021c), including anti-VGCC, DPPX, LGI1, CASPR2, and mGLUR1 Abs. Anti-GAD65 Ab impaired the cerebellar-mediated functions in vivo and disturbed GABA release in vitro, suggesting pathogenic roles in the manifestation of cerebellar ataxias (CAs). However, it is uncertain how anti-GAD65 Ab accesses to the GAD65 attached on the cytosolic face of vesicles (Mitoma et al. 2017).

While the clinical profiles and underlying immune mechanisms have diverse natures, common therapeutic strategies have been applied for IMCAs. This chapter is a review of the currently available immune therapies for each etiology and explains the common therapeutic strategies in IMCAs. We emphasize the need for early intervention to stop the progression of autoimmune processes during the period when the cerebellar reserve is preserved. We also argue that the therapeutic strategies in IMCAs can be generalized to other pathologies, such as degenerative CAs.

Table 1 Classification of autoantibodies in immune-mediated cerebellar ataxias

Characterized autoantibodies, suggestive of a specific etiology in IMCAs	
Well characterized	
Anti-gliadin, TG 2, 6	Gluten ataxia
Anti-Yo	PCDs; Breast, uterus, and ovarian carcinomas
Anti-Hu	PCDs; Small cell lung carcinoma
Anti-CV2	PCDs; Small cell lung carcinoma, thymoma
Anti-Ri	PCDs; Paraneoplastic OMS; breast carcinoma
Anti-Ma2	PCDs; Testis and lung carcinoma
Partially characterized	
Anti-Tr	PCDs; Hodgkin's lymphoma
Autoantibodies found in various neurological conditions (e.g., *CAs), suggestive of autoimmune pathomechanisms*	
Autoantibodies assumed to have pathogenic roles in the development of CAs	
Anti-VGCC (P/Q type)	Ca channel dysfunction: anti-VGCC ataxia, PCDs, Lambert-Eaton syndrome
Anti-DPPX	?K channel dysfunction: anti-DPPX ataxia, limbic encephalitis
Anti-LGI1	?K channel dysfunction and AMPA-R: anti-LGI1 ataxia, limbic encephalitis
Anti-CASPR2	?K channel dysfunction: anti-Caspr2 ataxia, limbic encephalitis, Morvan syndrome
Anti-mGluR1	mGluR dysfunction: anti-mGluR ataxia, PCDs
Anti-GAD65 (high titer)	Low GABA release: anti-GAD ataxia, PCDs, SPS
Anti-MAG	Although pathogenic for neuropathy, mechanism still uncertain: anti-MAG ataxia
Autoantibodies with unreported pathogenic actions	
Anti-thyroid	PACA, thyroid autoimmune diseases
Anti-SS_A(Ro), SS_B	PACA, Sjögren syndrome
Autoantibodies reported only in a few CA patients, with less characterized significance in ataxia	
Anti-ZIC4	Paraneoplastic (rare)
Anti-PCA2	Paraneoplastic (rare)
Anti-glycine R	Paraneoplastic OMS (rare)
Anti-GluRδ2	PIC; unknown
Anti-PKCγ	Reported in a few patients with neoplasm
Anti-Ca/ARHGAP26	Reported in a few patients with neoplasm; unknown
Anti-SOX1	Reported in a few patients with neoplasm; unknown.
Anti-CARP VIII	Reported in a few patients with neoplasm; unknown.
Anti-Homer3	Reported in a few patients with neoplasm; unknown
Anti-Sj/ITPR-1	Unknown
Anti-Septin-5	Unknown
Anti-Neurochondrin	Unknown
Anti-Nb/AP3B2	Unknown

(continued)

Table 1 (continued)

Source: Modified from Mitoma et al. (2021a)
Note: Unknown conditions might be PACA
Abbreviations: *AMPA-R* alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor, *CA* cerebellar ataxia, *Ca/ARHGAP26* Ca/Rho GTPase-activating protein 26, *CARP VIII* carbonic anhydrase-related protein VIII, *CASPR2* contactin-associated protein-like 2, *DPPX* dipeptidyl-peptidase-like protein 6, *GABA* gamma aminobutyric acid, *GAD65* glutamic acid decarboxylase 65, *GluRδ2* glutamate receptor delta2, *IMCAs* immune-mediated cerebellar ataxias, *LGI1* leucine-rich glioma-inactivated 1, *MAG* myelin associated glycoprotein, *mGluR1* metabotropic glutamate receptor, *Nb/AP3B2* Nb/adaptor complex 3 B2, *OMS* opsoclonus myoclonus syndrome, *PACA* primary autoimmune cerebellar ataxia, *PCA2* Purkinje cell antibody 2, *PCDs* paraneoplastic cerebellar degenerations, *PIC* post-infectious cerebellitis, *PKCγ* protein kinase C gamma, *Sj/ITPR-1* Sj/inositol 1,4,5-trisphosphate receptor 1, *SPS* stiff Person Syndrome, *SOX1* sex determining region Y-related high-mobility group box 1, *TG* transglutaminase, *VGCC* voltage gated calcium channel, *ZIC4* zinc finger protein of the cerebellum 4

2 Available Treatment Options for Each Subtype of IMCAs

2.1 IMCAs with Autoimmunity Triggered by Another Condition

2.1.1 Post-infectious Cerebellitis

PIC is defined as cerebellar inflammation induced by immune-mediated mechanisms triggered by viral or bacterial infection (Mitoma et al. 2016, 2021a, b, c; Hadjivassiliou et al. 2019). PIC affects mainly children after varicella infection (Mitoma et al. 2016, 2021a; Hadjivassiliou et al. 2019). PIC is characterized by acute-onset gait ataxia, associated with meningeal signs, sometimes increased intracranial pressure, with or without extracerebellar manifestations, such as temporary reduction in consciousness, seizures, altered mental status (e.g., extreme irritability), and extracerebellar focal signs (Connolly et al. 1994).

PIC is characteristically self-limiting. One large-scale study reported full recovery of CA in 72% of 60 pediatric patients within 2 months (Connolly et al. 1994). Accordingly, close observation of the patient is recommended (Hadjivassiliou et al. 2019). Intravenous acyclovir should be administered very early to be effective in case of PIC post-varicella infections. The efficacy of oral doxycycline, amoxicillin, and cefuroxime axetil for treating Lyme disease has been established in multiple trials (Sanchez et al. 2016). In severe central nervous system (CNS) infections, parenteral treatment with ceftriaxone is highly effective (Halperin 2014). Various combinations of immunotherapeutic agents are available but should only be used when the ataxia persists or progresses (Sawaishi and Takada 2002), including steroids, plasmapheresis, and immunoglobulins IV (IVIg).

2.1.2 Miller Fisher Syndrome

MFS is characterized by an acute-onset triad of ophthalmoplegia, ataxia, and areflexia (Fisher 1956). Autoimmunity is triggered by infection, similar to Guillain-Barré syndrome (GBS) (Hadjivassiliou et al. 2019). A large-scale study involving 50 consecutive patients showed that viral infection (usually respiratory) or bacterial (usually caused by *Campylobacter jejuni*) precedes the appearance of the above triad with a median interval of 8 days (Mori et al. 2001).

Since MFS shows a self-limiting clinical course, no immunotherapy is usually required (Hadjivassiliou et al. 2019). One large-scale study showed full recovery within 6 months in all patients (Mori et al. 2001). Notably, the speed of recovery and the outcome were the same in patients who were treated conservatively, compared to those who received either corticosteroids, IVIg, or plasmapheresis (Mori et al. 2001). Nevertheless, these last therapies are often administered.

2.1.3 Gluten Ataxia

GA is defined as sporadic CA associated with gluten sensitivity (Hadjivassiliou et al. 1998). The autoimmunity is triggered by the sensitivity to gluten found in wheat, rye, and barley. Gluten, a family of protein-containing grains, is composed of gliadin and glutenin. GA affects individuals in their forties and fifties and exhibits either chronic or insidious onset (Hadjivassiliou et al. 1998).

Gluten avoidance, by adhering to a gluten-free diet (GFD) regimen, is the first line of therapy for GA since it can eliminate antigens that trigger immune-mediated mechanisms, similar to the strategy used in Coeliac Disease (CD) (Hadjivassiliou et al. 1998, 2019; Mitoma et al. 2015). One large-scale study based on 43 patients showed improvement in CAs in patients who strictly adhered to GFD compared to those who did not (Hadjivassiliou et al. 2003). Clinical improvement correlated significantly with the severity of cerebellar atrophy and was evident in patients with mild ataxia, and gluten-free diet halted and stabilized CAs, although it did not improve clinical symptoms in some patients with severe ataxia (Mitoma et al. 2015).

In contrast to the above study, some case studies reported the effectiveness of immunotherapy (e.g., IVIg) in non-responders to GFD (Souayah et al. 2008). The lack of therapeutic benefits can be attributed to poor adherence to GFD or hypersensitivity to gluten, where small amounts of gluten present in commercially available gluten-free food or due to cross-contamination can cause strong autoimmune reactions perpetuating the cerebellar damage (Hadjivassiliou et al. 2019). In such cases, anti-gliadin Ab (AGA) and magnetic resonance (MR) spectroscopy can be used as markers for adherence to GFD (Hadjivassiliou et al. 2002, 2008b). Persistently high levels of AGA are present in the above two groups of patients (Hadjivassiliou et al. 2002, 2008a). In this regard, MR spectroscopy is useful as it shows an increasing ratio of N-Acetyl-Aspartate/Creatine (NAA/Cr) area in the cerebellar vermis in patients who adhere to GFD but not in those who do not (Hadjivassiliou et al. 2017a). When further dietetic review by an expert dietitian does not lead to

improvement in AGA titers and MR spectroscopy, switching to immunotherapy should be considered, including IVIg or immunosuppressants (e.g., mycophenolate mofetil, rituximab, and cyclophosphamide) (Mitoma et al. 2015; Hadjivassiliou et al. 2019).

Myoclonic ataxia with refractory celiac disease is a subtype of gluten sensitivity-related autoimmune disorder with resistance to GFD (Sarrigiannis et al. 2014). Although the myoclonus is of cortical origin, hyperexcitability of the cerebral cortex is elicited by cerebellar dysfunction (Sarrigiannis et al. 2014). The refractoriness in myoclonic ataxia is associated with residual enteropathy, which is detected by repeat duodenal biopsies (Hadjivassiliou et al. 2019). GFD plus immunosuppression, usually with mycophenolate, is used in such patients, in addition to cladribine and anti-epileptic drugs to control the myoclonus (Hadjivassiliou et al. 2019). However, the prognosis is poor in such patients.

2.1.4 Opsoclonus Myoclonus Syndrome

OMS has diverse autoimmune background, including post-infectious, idiopathic, and paraneoplastic (Bataller et al. 2001; Klaas et al. 2012; Armangué et al. 2016). OMS affects mainly children, and is associated with neuroblastoma (Bataller et al. 2001; Klaas et al. 2012; Armangué et al. 2016). Opsoclonus is characterized by involuntary repetitive, random, and rapid eye movements in both horizontal and vertical directions, compared with action myoclonus, which is characterized by irregular and jerky movements primarily in upper limbs (Hadjivassiliou et al. 2019). Since OMS is usually associated with ataxic syndromes, this condition is termed opsoclonus myoclonus ataxia syndrome (Hadjivassiliou et al. 2019). The presenting symptom is acute vertigo or subacute ataxia with myoclonus.

Post-infectious OMS and some idiopathic OMS are self-limiting. If symptoms persist, immunotherapy should be introduced (Bataller et al. 2001; Klaas et al. 2012; Armangué et al. 2016). In case of paraneoplastic OMS, any associated neoplasm should be removed first, if possible, followed by a combination of immunotherapies including corticosteroids, IVIg, plasmapheresis, immunosuppressants, and rituximab (Bataller et al. 2001; Klaas et al. 2012; Armangué et al. 2016). Prognosis is better in post-infectious OMS and idiopathic OMS than in paraneoplastic OMS (Bataller et al. 2001; Klaas et al. 2012; Armangué et al. 2016). One large-scale study showed good response, defined as recovery of the modified Rankin Score to ≤ 2, in 84% of patients with idiopathic OMS and 39% of patients with paraneoplastic OMS, and that relapse occurred in 7% of those with idiopathic OMS compared with 24% of patients with paraneoplastic OMS (Armangué et al. 2016).

2.1.5 Paraneoplastic Cerebellar Degeneration

PCD represents cerebellar degeneration induced by cancer-related autoimmunity (Mitoma et al. 2016; Dalmau and Rosenfeld 2008; Muñiz-Castrillo and Honnorat 2019). The characteristic onconeural autoantibodies correlate with a specific type of

associated neoplasm (Mitoma et al. 2016; Dalmau and Rosenfeld 2008; Muñiz-Castrillo and Honnorat 2019). PCD shows acute or subacute onset and is sometimes preceded by prodromal clinical symptoms, such as nausea, vomiting, and dizziness, resembling viral infection-related diseases (Dalmau and Rosenfeld 2008; Muñiz-Castrillo and Honnorat 2019).

The first line of therapy is to remove the underlying neoplasm by the combinations of surgery and radiochemotherapy to prevent metastasis and remove antigen(s) triggering the autoimmunity (Dalmau and Rosenfeld 2008; Muñiz-Castrillo and Honnorat 2019). Immunotherapy should be the next line of treatment following the anti-cancer therapy (Dalmau and Rosenfeld 2008; Muñiz-Castrillo and Honnorat 2019). The recommended immunotherapy is the combination of IVIg or plasmapheresis with cyclophosphamide (Dalmau and Rosenfeld 2008).

Although aggressive chemotherapy and immunotherapy have been attempted and therapeutic benefits have been reported in some case reports, the prognosis remains poor (Mitoma et al. 2016; Dalmau and Rosenfeld 2008; Muñiz-Castrillo and Honnorat 2019). A study of 22 patients positive for anti-Yo Ab reported therapeutic improvement in less than 10% of the trials (Peterson et al. 1992). Another study reported no benefits in 23 patients with PCD (Rojas et al. 2000). Long-term studies on survival time reported a mean survival time from the onset of symptoms of 42 months in 63 patients with paraneoplastic neurological syndrome (Candler et al. 2004), and a median survival time from the first therapy of 10.2 months in 16 patients with PCD (Keime-Guibert et al. 2000). Notably, prognosis varied among the associated onconeural autoantibodies: the median survival time from diagnosis was 113 months in patients positive for anti-Tr Ab (some patients may enter in long-term remission), >69 months in those positive for anti-Ri antibody, 13 months in those positive for anti-Yo antibody, and 7 months in those positive for anti-Hu antibody (Shams'ili et al. 2003).

Currently, clinical trials aimed at stopping the progressive autoimmune insults are being conducted. In one such trial, the combination of IVIg or plasmapheresis with cyclophosphamide was reported to be effective in a subgroup of patients (Dalmau and Rosenfeld 2008).

2.2 IMCAs with Autoimmunity Not Triggered by Another Condition

2.2.1 Anti-GAD Ataxia

Glutamic acid decarboxylase (GAD) is an enzyme that catalyzes the conversion of glutamate to GABA (Honnorat et al. 2001; Mitoma et al. 2017; Graus et al. 2020; Dade et al. 2020). GAD has two isoforms: GAD65 and GAD67. Autoantibodies against GAD65 are associated with CA (Honnorat et al. 2001; Mitoma et al. 2017; Graus et al. 2020; Dade et al. 2020). Serum and cerebrospinal fluid (CSF) titers of anti-GAD65 Ab are higher in anti-GAD ataxia; usually, more than 10,000 U/mL (or 10- to 100-fold higher) compared to those of patients with type 1 diabetes mellitus

(T1DM) (Honnorat et al. 2001; Mitoma et al. 2017; Graus et al. 2020; Dade et al. 2020). The pathogenic role of anti-GAD65 Ab in the development of CAs remains a subject of debate. Doubt on the direct pathogenic role of anti-GAD Ab is based on its cytoplasmic location (Graus et al. 2020). However, intracerebellar administration of patients' CSF IgGs elicits ataxic symptoms in in vivo preparations (Manto et al. 2015). Furthermore, in vitro studies involving the use of cerebellar slices show that CSF IgGs decrease GABA release, an action abolished after absorption of anti-GAD Ab with recombinant GAD65 and the use of GAD65 knock-out mice slices (Mitoma et al. 2017). The above effects described in in vivo and in vitro studies are epitope-dependent (Mitoma et al. 2017). These physiological studies suggest that anti-GAD65 Ab acts on GABA neurons to suppress GABA release, leading to the manifestation of CAs. It should be acknowledged that these physiological studies do not exclude the involvement of cell-mediated mechanisms.

The triggering factor of this autoimmunity is not apparent. Anti-GAD ataxia can be associated with other types of IMCAs, such as PCD and GA (Graus et al. 2020). A study on 50 patients with anti-GAD65 Ab showed that 35 patients were gluten sensitive, and GFD improved their CAs (51% of the 35 patients) or stopped the progression (37%), suggesting an overlap between GA and anti-GAD65 (Hadjivassiliou et al. 2021). While autoimmunity toward GAD65 affects the entire CNS, the cerebellum is one of the most vulnerable areas (Mitoma et al. 2017; Graus et al. 2020). Anti-GAD ataxia affects mostly women in their 60s and exhibits either subacute or chronic/insidious onset (Honnorat et al. 2001; Ariño et al. 2014).

The aim of induction therapy is to minimize the progression of CA, while that of maintenance therapy is to prevent relapse (Mitoma et al. 2015). Both types of therapies include corticosteroids, IVIg, immunosuppressants, plasmapheresis, and rituximab, either alone or in combination (Mitoma et al. 2015; Ariño et al. 2014). However, there are no studies that have compared the differences among these therapeutic options (Mitoma et al. 2015). Some patients responded well to immunotherapies, with clinical improvement in CA correlating well with falls in Ab titers (Mitoma et al. 2015). The prognosis is better in the subacute type than in the chronic type of anti-GAD ataxia (Ariño et al. 2014). Notably, there have been accumulated case reports suggesting therapeutic benefits of rituximab (Mitoma et al. 2015). In anti-GAD65 Ab positive-limbic encephalitis and stiff-person syndrome, it was reported that the treatment with high-dose corticosteroids, IVIg, and plasmapheresis had minimal response, but treatment escalation with rituximab and cyclophosphamide was associated with clinically significant improvements, reducing antibody titers and resolution of magnetic resonance imaging (MRI) changes (Triplett et al. 2018; Katoh et al. 2010). Taking possible pathogenic roles of anti-GAD65 Ab into consideration, rituximab therapies might be possible effective therapeutic strategies. Further studies are needed to elucidate the significance of anti-GAD Ab.

2.2.2 Primary Autoimmune Cerebellar Ataxia

Despite the clinical profile spectrum of PACA (e.g., subacute onset, history of autoimmune disease, predominant gait ataxia, association of autoantibodies, and good benefits from immunotherapies), a subgroup of patients present with features that do not meet the criteria of the above common subtypes (Hadjivassiliou et al. 2008a, b, 2020). Thus, the spectrum of PACA serves as an umbrella that covers heterogeneous etiologies (Hadjivassiliou et al. 2008a, b, 2020). Consistently, various types of less characterized autoantibodies are associated with PACA in some patients (Table 1) and immunohistochemical studies show various staining patterns for autoantibodies in about 60% of the patients (Hadjivassiliou et al. 2008a, b).

Many types of immunotherapies have been used in PACA. A recent study on 30 patients with PACA showed improvements in MR spectroscopy in 22 patients who received mycophenolate and a strong correlation between improvement in MR spectroscopy and Scale of the Assessment and Rating of Ataxia (SARA) (Hadjivassiliou et al. 2020).

3 General Principles in Therapeutic Strategies

Figure 1 summarizes the response to immunotherapy and recovery. We discuss here the possible mechanisms underlying the therapeutic outcomes.

3.1 The Response to Immunotherapy

When immunotherapy can stop disease progression, the clinical course can be classified into three patterns, depending on the extent of recovery: (***a***) full recovery, (***b***) partial recovery, and (***c***) stabilization (Fig. 1a). On the other hand, three types of clinical courses have been used to describe the lack of response to immunotherapy and progression of the disease: (***d***) relapse, (***e***) gradually progressive, and (***f***) stabilization (Fig. 1a). Notably, each etiology shows a specific pattern among the above six patterns. PIC, MFS, and post-infectious OMS usually show good prognosis (self-limiting or good response to immunotherapies) (type ***a***). In GA and subacute anti-GAD ataxia, gluten-free diet and/or immunotherapy are mostly effective (types ***b*** and ***c***). In contrast, paraneoplastic OMS, PCD, myoclonic ataxia with refractory celiac disease, and chronic anti-GAD ataxia show resistance to immunotherapy (types ***d***, ***e***, and ***f***).

One possible explanation for the differences in the response to immunotherapy is the extent of antigen exposure. Post-infectious conditions are characterized by transient exposure to the antigens. In contrast, paraneoplastic conditions are associated with persistent exposure to the antigens. Chen and Mellman proposed the concept of the cancer-immunity cycle (Chen and Mellman 2013). This cycle includes the

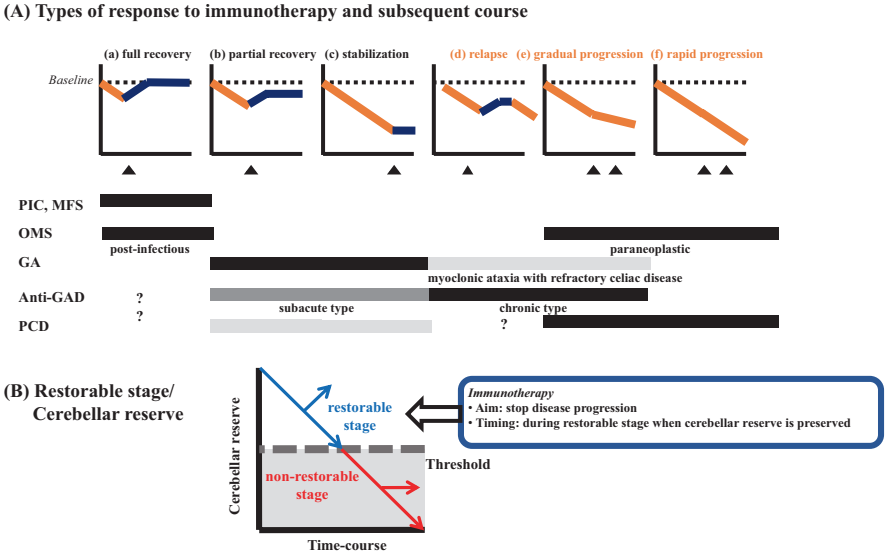

Fig. 1 (**a**) Six patterns of the clinical courses of immune-mediated cerebellar ataxias: (*a*) full recovery, (*b*) partial recovery, (*c*) stabilization, (*d*) relapse, (*e*) gradually progressive, and (*f*) rapidly progressive. PIC, post-infectious cerebellitis; OMS, opsoclonus myoclonus syndrome; anti-GAD, anti-GAD ataxia; PCD, paraneoplastic cerebellar degeneration. (**b**) Schematic diagram of the concept of cerebellar reserve. (Modified from Mitoma et al. 2015)

following events: (1) release of neoantigens created by oncogenesis, (2) presentation of these antigens on MHCI and MHCII molecules by dendritic cells (DCs), (3) priming and activation of effector T cells, (4) trafficking of cytotoxic T cells (CTLs) to cancer sites, (5) infiltration of CTLs into cancer tissues, (6) recognition of cancer cells by CTLs through the interaction between T-cell receptor (TCR) and the cognate antigens bound to MHC, and finally (7) killing of cancer cells. The killing of tumor cells releases additional cancer antigens, resulting in the continuity of the cancer-immunity cycle. The cancer-immunity cycle indicates that the persistent autoimmune stimulus can cause persistent augmentation and amplification of autoimmune attacks.

3.2 Subsequent Recovery and Cerebellar Reserve

Functional reversibility can be also attributed to the specific capacity of the cerebellum for compensation and restoration following the immune insult. We call this capacity the cerebellar reserve (Mitoma et al. 2020). Various forms of synaptic plasticity and redundant mossy fiber-mediated inputs constitute the cerebellar reserve (Mitoma et al. 2021b). Notably, the extent of progression of the pathophysiological mechanisms determines the degree of reversibility, suggesting the existence of a

threshold (Fig. 1b). Although various immunotherapies can arrest the progression of cerebellar tissue damage under this threshold, such intervention may not be accompanied by clinical improvement. Thus, immunotherapy should be introduced during the period when cerebellar reserve is above this threshold. Such threshold can be understood as the limit required to obtain a sufficient level of activity in the cerebellar circuitry. Above the threshold, rehabilitation, non-invasive cerebellar stimulation (NICS), or theoretically neurotransplantation effectively reorganize the lost cerebellar function (Ilg et al. 2010; Manto et al. 2021; Cendelin et al. 2019).

Hadjivassiliou et al. (2017b) investigated the prevalence of IMCAs in 1500 UK patients with progressive ataxia (Hadjivassiliou et al. 2017a, b). The authors reported that 30% of the patients had familial/genetic ataxia, although some did not show evidence of family history, and that 9% of the patients had cerebellar variant of multiple systemic atrophy (MSA-C) (prevalence out of total progressive ataxic cases). Apart from patients with the above degenerative CA, which account for 39% of the surveyed population, 25% had definite IMCAs, 20% had GA, and 2% had PCD, while 2% had anti-GAD ataxia, 1% had PIC, and <1% had OMS. Interestingly, 19% of the patients were classified as idiopathic sporadic ataxia (the cause was unknown). This category might include patients with IMCAs (Hadjivassiliou et al. 2017b).

The above study suggests that some patients with IMCAs are overlooked, and therapeutic opportunity is missed, mainly when the autoimmunity is not triggered by other conditions or the associated autoantibodies are not well characterized (Table 1). We argue that every effort should be made to reduce the diagnostic delay and to initiate early therapy to avoid the risk of transition from a treatable state to an irreversible condition with the associated accumulation of disability (Mitoma et al. 2018).

4 Conclusion

The response to immunotherapy depends on the underlying etiology in IMCAs; PIC, MFS, and post-infectious OMS are self-limiting or respond well to immunotherapy, while PCD shows resistance to immunotherapy. Antigenic stimulation is transient in infectious conditions, but persistent in paraneoplastic conditions due to the cancer-immunity cycle. Thus, the extent of antigen stimulation is a likely factor in modulating the response to immunotherapy. When immunotherapy can stop autoimmune progression during the period when the cerebellar reserve is preserved, i.e., the capacity for compensation and restoration to pathologies, CAs could partially or fully recover following functional reorganization. Thus, clinicians should assess and identify the therapeutic opportunity as early as possible.

This therapeutic strategy could be applicable also in other cerebellar pathologies, such as degenerative CA. Molecular targeting therapies that can stop or slow disease progression are promising in the near future. In line with the management of IMCAs, these novel methods should be introduced while the cerebellar reserve is preserved.

Thus, the control of disease-specific pathology and reinforcement of cerebellar reserve are the two main therapeutic targets in cerebellar diseases.

Conflicts of Interest The authors declare no conflict of interest.

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Coenzyme Q10 in Multiple System Atrophy

Jun Mitsui and Shoji Tsuji

Abstract Multiple system atrophy (MSA) is a progressive neurodegenerative disease characterized by autonomic failure in addition to various combinations of parkinsonism, cerebellar ataxia, and pyramidal dysfunction. We previously showed that several patients from multiplex families with MSA carried biallelic variants in *COQ2*; furthermore, the carrier frequency of the V393A variant in *COQ2* was significantly higher in patients with sporadic MSA than that in controls in Japan. Subsequent replication studies were conducted mainly in East Asia, and a meta-analysis integrating these results finally established *COQ2* as a susceptibility gene for MSA. Furthermore, lower levels of coenzyme Q10 (CoQ10) in the blood, cerebrospinal fluid, fibroblasts, and cerebellar tissues have been reported in patients with MSA, than in controls. These results may suggest that CoQ10 supplementation could prove to be a therapeutic intervention in MSA.

Keywords Multiple system atrophy · Multiplex family · *COQ2* · Coenzyme Q10 · Ubiquinol · Registry

1 An Overview of Multiple System Atrophy

Multiple system atrophy (MSA) is a progressive neurodegenerative disease characterized by autonomic failure in addition to various combinations of parkinsonism, cerebellar ataxia, and pyramidal dysfunction. MSA has been subdivided into two

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_28

main distinct subtypes: MSA-cerebellar (C) and MSA-parkinsonism (P) (Gilman et al. 2008). Oligodendroglial cytoplasmic inclusions, whose main constituent is misfolded α-synuclein (Wakabayashi et al. 1998), are the cardinal neuropathologic hallmark of MSA. MSA is a neurodegenerative disease that is extremely disabling with the mean survival of 6–10 years from the onset of symptoms (Watanabe et al. 2002; Schrag et al. 2008; Kim et al. 2011; Low et al. 2015; Wenning et al. 2013). Therefore, it is urgent to develop therapeutic interventions to suppress the disease progression of symptoms and ultimately to prolong the life expectancy. Despite preclinical evidence supporting neuroprotective effects, clinical trials employing riluzole (Bensimon et al. 2009), minocycline (Dodel et al. 2010), rifampicin (Low et al. 2014), rasagiline (Poewe et al. 2015), and epigallocatechin-gallate (Levin et al. 2019) have shown no therapeutic benefit for MSA thus far.

The distribution of the clinical subtypes of MSA (MSA-P and MSA-C) varies widely among populations, although patient selection bias may be involved depending on the clinical practice in each region. MSA-P is more frequently seen in North American (Low et al. 2015; Roncevic et al. 2014) and European populations than MSA-C (Wenning et al. 2013; Rey et al. 2014; Ronchi et al. 2016). In Latin America, MSA-P is similarly more frequent in European descents, while MSA-C is predominant in Mestizo population (Gatto et al. 2014). In East Asia, MSA-C is more frequently seen in Japan (Watanabe et al. 2002; Yabe et al. 2006; Matsushima et al. 2021), Korea (Seo et al. 2010), and China (Guo et al. 2013; Li et al. 2021; Cao et al. 2014). Ozawa et al. performed semi-quantitative assessments of neuronal cell loss at 24 anatomical sites in MSA patients from the UK and Japan using autopsied brain materials and concluded that olivopontocerebellar-predominant pathology was significantly more frequent in the Japanese series than in the British series (Ozawa and Onodera 2017).

2 Multiplex Family with Multiple System Atrophy

On the basis of the findings that none of the relatives of 38 autopsy-confirmed MSA cases had been diagnosed as having MSA (Wenning et al. 1993), MSA has been widely considered to be a nongenetic disorder. Several recent epidemiological and genetic studies, however, have suggested the involvement of genetic factors in the development of MSA. Although very rare, several multiplex families with MSA have been reported (Hara et al. 2007; Soma et al. 2006; Wüllner et al. 2009; Fujioka et al. 2014; Hohler and Singh 2012). Some families have affected individuals in different generations, suggesting an autosomal dominant form of inheritance (Soma et al. 2006; Wüllner et al. 2009; Fujioka et al. 2014), whereas others have only affected siblings, suggesting an autosomal recessive form of inheritance (Hara et al. 2007; Hohler and Singh 2012). Hara et al. described the clinical features of 8 patients in 4 multiplex families with MSA with ages at onset ranging from 58 to 72 years (Hara et al. 2007). Two siblings in each family had MSA, suggesting an autosomal recessive form of inheritance. Consanguineous marriage was observed in

one of the four families. The most frequent phenotype was MSA-P, which was observed in five patients. Among the eight patients, one had autopsy-proven definite MSA, five had probable MSA, and two had possible MSA.

3 Discovery of *COQ2* as a Genetic Factor for Familial Multiple System Atrophy

A parametric linkage analysis was performed on six multiplex families with MSA, including the four families described by Hara et al. (2007), which revealed no single locus showing a linkage compatible with autosomal recessive inheritance. However, in the parametric linkage analysis allowing for the heterogeneity, we detected several loci showing positive scores for heterogeneity logarithm of the odds (HLOD), suggesting that more than one locus was involved in the different multiplex families. In particular, two regions on chromosome 4 showed the highest HLOD scores. Parametric linkage analysis of chromosome 4 in individual pedigrees revealed positive LOD scores in an overlapping region in the four families. One of these families, in which both parents were in a consanguineous marriage and the diagnosis of affected individuals was confirmed by autopsy, had the highest LOD score. Thus, we selected this family to undergo whole-genome sequence analysis. Whole-genome sequence analysis of an affected individual of the 2 affected members of this family generated 187.5 Gb of short reads, with 3,492,429 single-nucleotide variants (SNVs) and insertions/deletions. We winnowed the 3,492,429 variants down to 4 by selecting SNVs that were located in the candidate regions defined by the linkage analysis, which were predicted to cause amino acid changes, and that were not registered in the database of single-nucleotide polymorphisms, build 130 (dbSNP130), indicating that the 4 variants are rare in the general population. We then examined the allele frequencies of the 4 candidate variants in 180 Japanese control subjects. Three had minor allele frequencies above 1%, and only M128V in *COQ2* (NM_015697) was not observed in the 180 control subjects. We therefore considered homozygous M128V in *COQ2* as a candidate variant that confers susceptibility to familial MSA. Nucleotide sequence analysis of *COQ2* was further performed in five other multiplex families with affected siblings, and we found the compound heterozygous variants of R387*/V393A in *COQ2* in the two affected members of another family. The results suggest that *COQ2* confers susceptibility to familial MSA, albeit in only two of the six families.

COQ2 maps to chromosome 4q21 and comprises seven exons. Genomic DNA analysis showed that the first exon contains four potential in-frame ATG initiation codons (ATG1–ATG4) in the longest transcript NM_015697. Since the widely used amino acid numbering for the COQ2 protein follows the rule of indicating the most upstream ATG (ATG1) as the first codon, V393A variant is based on this numbering. Recent transcriptome and functional analyses of human *COQ2*, however, indicate that the most downstream ATG (ATG4) is the principal translation initiation

codon (NM_001358921) (Desbats et al. 2016). *COQ2* encodes an enzyme involved in the biosynthesis of coenzyme Q10 (CoQ10). It has been reported as one of the causative genes for primary CoQ10 deficiencies that manifested as fatal infantile multiorgan disease including encephalopathy and nephropathy (Quinzii et al. 2006). We confirmed that the level of CoQ10 in the autopsied cerebellum of a patient from a multiplex family with MSA carrying biallelic variants of *COQ2* was substantially decreased (The Multiple-System Atrophy Research Collaboration 2013).

4 Discovery of *COQ2* as a Genetic Risk Factor for Sporadic MSA

The next question was whether *COQ2* was also associated with the development of sporadic MSA. To investigate the involvement of *COQ2* variants in sporadic MSA, we conducted nucleotide sequence analysis of *COQ2* in a Japanese series consisting of 363 MSA patients and 520 controls (The Multiple-System Atrophy Research Collaboration 2013). Four patients with MSA simultaneously carried two variants (one carried I147T and V393A, one had R387Q/V393A, and two had V393A/V393A), whereas none of the controls had two variants of *COQ2*. We found that 29 patients with MSA and 17 controls were heterozygous for the V393A variant. In addition, we detected four singleton variants: two in patients with MSA (P157S and S163F) and two in controls (P72L and N386H). Of the *COQ2* variants, the V393A variant is relatively common in the Japanese population. We found that V393A was observed in 35 of 726 alleles (4.8%) from Japanese patients with MSA and in 17 of 1040 alleles (1.6%) from Japanese controls (odds ratio of the V393A allele for MSA, 3.05; 95% confidence interval [CI], 1.65–5.85; $p = 1.5 \times 10^{-4}$). Genotyping of *COQ2* in the second series of 2383 Japanese controls showed that V393A had an allele frequency of 2.2% (106 of 4766 alleles; odds ratio, 2.23; 95% CI, 1.46–3.32; $p = 6.0 \times 10^{-5}$). Genotyping of *COQ2* in Japanese individuals with other neurodegenerative diseases revealed that the allele frequencies of V393A were 2.0% (109 of 5456 alleles) among patients with Alzheimer disease, 2.5% (33 of 1318 alleles) among those with Parkinson's disease, and 2.4% (31 of 1268 alleles) among those with amyotrophic lateral sclerosis. These allele frequencies did not differ significantly from those in the first or second set of controls, confirming the specificity of the V393A variant in patients with MSA.

We then extended genotyping of *COQ2* in the European and North American series, consisting of 223 patients with MSA and 315 controls, and 172 patients with MSA and 294 controls, respectively. (The Multiple-System Atrophy Research Collaboration 2013) In the European series, we found four singleton *COQ2* variants (F79L, S1077T, T317A, and S347C) among the patients, whereas none of the controls had any variants in *COQ2*. In the North American series, we found two singleton *COQ2* variants (P99H in a patient with MSA and R119H in a control). Intriguingly, V393A, a relatively common variant in the Japanese population, was

neither found in patients with MSA nor in controls in either the European or North American series. To determine the functional effect of each variant on the mitochondrial aerobic energy production in which CoQ10 plays an essential role in electron transfer, we carried out functional complementation analysis by transforming the yeast *coq2*-null strain with nonmutated or mutated human *COQ2* cDNA (The Multiple-System Atrophy Research Collaboration 2013). Transformants of the BY4741 Δ*coq2* yeast strain with the mutated human *COQ2* cDNA with P99H, S107T, I147T, R119H, M128V, M128V-V393A, P157S, S163F, T317A, S347C, R387Q, or R387* showed substantially lower growth rates than those expressing nonmutated human *COQ2* cDNA. The transformants with mutated human *COQ2* cDNA with V66L, P72L, F79L, N386H, or V393A showed growth rates similar to those of the transformants expressing nonmutated human *COQ2* cDNA. We concluded that nine variants (P99H, S107T, R119H, I147T, P157S, S163F, T317A, S347C, and R387Q) were mildly or severely deleterious. Taken together with all the 3 series, 8 functionally impaired variants were identified in 758 patients with MSA, whereas only 1 variant was found in 1129 controls (odds ratio, 11.97; 95% CI, 1.60–531.52; $p = 0.004$), further supporting that functionally impaired *COQ2* variants are associated with sporadic MSA.

As mentioned above, V393A was not found in the European population, but only in the Japanese population. Subsequent association studies of *COQ2* in MSA also confirmed that V393A was not found in populations of European descents (Ronchi et al. 2016; Ogaki et al. 2014), whereas V393A was found in populations from East Asia, including Japan and China. Meta-analysis integrating the results of association studies in East Asia unequivocally established *COQ2* as a susceptibility gene for MSA (Zhao et al. 2016; Porto et al. 2021). A study in which the *coq2*-deficient yeast was transformed with mutated human *COQ2* cDNA with V393A showed a reduced basal oxygen consumption rate compared with that transformed with wild-type *COQ2* cDNA, indicating that V393A is a functionally impaired variant (Yasuda et al. 2019).

A study in which ATP levels were measured in multiple regions of MSA and control brains showed a reduction in ATP levels in disease-affected regions of MSA brains (Hsiao et al. 2019). A study using induced pluripotent stem cell (iPSC)-derived neurons from patients with MSA has been carried out (Nakamoto et al. 2018). This study showed decreased intracellular levels of CoQ10, decreased basal oxygen consumption rates, increased ratios of CellROX Green (probe for measuring oxidative stress)-positive neurons, and increased ratios of cleaved-caspase 3 (probe for measuring apoptosis)-positive neurons in iPSC-derived neurons from a patient with MSA carrying R387*/V393A in *COQ2* compared with those from control subjects. Note that the apoptosis rate also tended to be higher in iPSC-derived neurons from a patient without *COQ2* variants than in those from control subjects. In a study using cultured fibroblasts (Monzio Compagnoni et al. 2018), spectrophotometric analysis showed a reduced activity of complex II, and quantification of respiratory chain complexes by Western blot analysis showed a general reduction in the levels of complexes II, III, and IV in the cultured fibroblasts from MSA patients compared with those from controls. Intriguingly, decreases in CoQ10 levels have been

observed in the blood (Kasai et al. 2016; Mitsui et al. 2016; Du et al. 2018), cerebrospinal fluid (Compta et al. 2018), cultured fibroblasts (Monzio Compagnoni et al. 2018), and cerebellar tissues (Schottlaender et al. 2016; Barca et al. 2016) of patients with MSA who do not carry variants in *COQ2*, but the reason for this is not unveiled. In addition to the *COQ2* variants, several unidentified genetic and environmental factors may well be involved in the reduction of CoQ10 levels in MSA. These findings indicate that decreased CoQ10 levels commonly occur in patients with MSA and may underlie the pathogenesis of MSA, although the exact mechanisms responsible for the decreased CoQ10 levels remain to be elucidated.

Thus, a series of studies have established that *COQ2* is associated with the development of not only familial but also sporadic MSA, and that decreased CoQ10 levels commonly occur in MSA patients regardless of the *COQ2* genotype, leading to an idea that decreased CoQ10 levels play an essential role in the pathogenesis of MSA and that CoQ10 supplementation may be efficacious for preventing the progression of MSA.

5 Coenzyme Q10

Functions of CoQ10

CoQ10 is a derivative of benzoquinone and has a long isoprenyl side chain (10 refers to the number of isoprenyl subunits in humans), which is retained in the membrane owing to its hydrophobic property. There are three redox states of CoQ10: fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol). CoQ10 is well recognized as a key component in the mitochondrial electron transport. Its functions as a two-electron carrier to move between ubiquinone and ubiquinol, and as a one-electron carrier to move between ubisemiquinone and other forms, are central to its role in the electron transport chain through iron–sulfur clusters in the mitochondria, which can only accept one electron at a time. Specifically, CoQ10 shuttles electrons from complexes I and II to complex III of the mitochondrial respiratory chain (Fig. 1). CoQ10 is also a potent antioxidant, preventing the generation of free radicals and modifications of proteins, lipids, and DNA. The antioxidant function of CoQ10 is attributed to its reduced ubiquinol form, which must be constantly regenerated from its oxidized form, ubiquinone (Fig. 1).

Biosynthesis of CoQ10

The biosynthesis of the CoQ10 molecule involves three major steps. The first step is the synthesis of a benzoquinone structure from 4-OH-benzoate derived from tyrosine or phenylalanine. The second step is synthesis of a polyisoprenoid side chain from acetyl-CoA via the mevalonate pathway (*PDSS1/PDSS2*) followed by the synthesis of decaprenyl-4-OH-benzoate from 4-OH-benzoate and decaprenyl-diphosphate (*COQ2*). The third step is the synthesis of CoQ10 with modifications of the ring structure of benzoquinone, such as hydroxylation, methylation, and

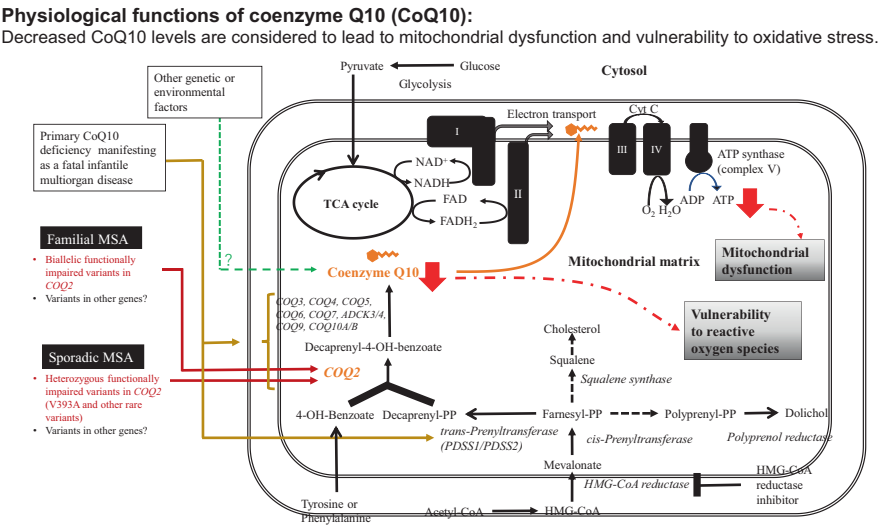

Fig. 1 The biosynthetic pathway of CoQ10 along with the glycolytic pathway and the electron-transfer system in mitochondria. Biallelic functionally impaired *COQ2* variants have been identified in patients from multiplex families with MSA and the heterozygous V393A variant in *COQ2* has been shown to be associated with sporadic MSA in East Asian populations. Recent investigations have further demonstrated that CoQ10 levels are decreased in plasma, cultured fibroblasts, cerebrospinal fluid, and autopsied cerebellum of patients who do not carry the V393A variant in *COQ2*. Taken together, decreased CoQ10 levels leading to decreased ATP production in mitochondria and vulnerability to oxidative stress underlie the pathogenetic mechanisms in MSA, and furthermore, it is expected that supplementation of CoQ10 contributes to suppressing disease progression of MSA patients

decarboxylation, to form CoQ10 (*COQ3*, *COQ4*, *COQ5*, *COQ6*, COQ7, *ADCK3/4*, *COQ9*, and *COQ10A/B*) (Fig. 1) (Hargreaves et al. 2020).

Bioavailability of CoQ10

Being a lipid-soluble substance, the absorption of CoQ10 follows the same process as that of lipids in the gastrointestinal tract, in which micellization occurs in the duodenum, and absorption occurs in the small intestine. CoQ10 molecules entering into enterocytes are incorporated into chylomicrons and enter the lymphatic circulation, where they travel through the thoracic lymphatics and eventually enter the subclavian vein and the blood circulation, in which CoQ10 molecules are present predominantly in the reduced form. The bioavailability of ubiquinol was reported to be about twice as high as that of ubiquinone (Hosoe et al. 2007). Tissues with high-energy requirements such as the heart, kidney, liver, brain, and muscle contain relatively high levels of CoQ10. In the subcellular distribution of CoQ10, a large portion (40–50%) of CoQ10 is localized in the mitochondrial inner membrane, with smaller amounts in other organelles and the cytosol (Bhagavan and Chopra 2006; Mantle and Dybring 2020). Regarding the permeability of CoQ10 through the blood-brain barrier (BBB), a study in which 1200 mg of ubiquinol was administered to a patient

from a multiplex family with MSA showed an increase in total CoQ10 in the cerebrospinal fluid from the basal level of 0.22×10^{-3} μg/mL up to 14.06×10^{-3} μg/mL after administration of 1200 mg of ubiquinol, supporting the penetrance of ubiquinol through BBB (Mitsui et al. 2017).

6 Toward Clinical Trials of Drugs for MSA

On the basis of progress of the basic research aforementioned, we hypothesized that the decreased levels of CoQ10 are involved in the pathogenesis of MSA, and that CoQ10 supplementation delays the progression of MSA. We have started to conduct clinical trials of CoQ10 as an investigational drug for MSA.

Registry and Natural History

Since MSA is a rare disease affecting approximately 12,000 patients in Japan, recruitment of a sufficient number of patients to participate in clinical trials is challenging. To overcome this difficulty, we launched a multicenter-based patient registry for MSA in Japan (https://msajp.org/). In this registry, neurologists at participating institutions will register patients at their request. In addition to its use in recruitment for clinical trials, we ask participants to contribute to genetic and natural history studies of MSA with informed consent. All clinical information and bioresources (genomic DNA, plasma, and lymphoblastoid cell lines) have been deposited in the Intractable Disease Research Resource Bank (https://raredis.nibiohn.go.jp/) operated by the National Institutes of Biomedical Innovation, Health and Nutrition in Japan. Researchers conducting studies on MSA can request for clinical information and bioresource samples for use in their research from this biobank. As of October 2021, the cumulative number of registrations has exceeded 500.

Regarding the natural history of patients, disease progression was assessed using the Japanese version of the Unified Multiple System Atrophy Rating Scale (UMSARS) (Chikada et al. 2021). A telephone interview of activities of daily living (UMSARS part 1) by a nurse is conducted every 6 months, and a motor function evaluation (UMSARS part 2) by neurologists is conducted every 12 months. Prospective studies of the natural history of MSA using the UMSARS scores have been carried out in the United States and Europe (Low et al. 2015; Wenning et al. 2013), but not fully conducted in Japanese MSA patients, where the relative frequency of disease subtypes substantially differs from those in European or North American populations. Preliminary analysis using this registry showed that the changes at 12 months in UMSARS part 2 scores in the Japanese MSA cases are similar to those previously reported (Low et al. 2015; Wenning et al. 2013). Also consistent with previous reports (Low et al. 2015; Wenning et al. 2013), 12-month changes in UMSARS part 2 scores are inversely correlated with baseline UMSARS part 2 scores.

Japanese Translation of the Unified Multiple System Atrophy Rating Scale (UMSARS)

The European MSA Study Group developed the UMSARS in 2004 to measure the clinical severity applicable to all the subtypes of MSA (Wenning et al. 2004). UMSARS part 1 is used to score neurological symptoms and autonomic dysfunctions with 12 items rated on a scale of 0 (normal) to 4 (extremely disabled). UMSARS part 2 is used for motor examination with 14 items also rated on a scale of 0 (normal) to 4 (extremely impaired). This scale was shown to be a reliable and valid measurement of the clinical severity of MSA and has been broadly used as the assessment tool in clinical trials (Dodel et al. 2010; Low et al. 2014; Poewe et al. 2015; Levin et al. 2019).

To employ UMSARS in clinical trials of drugs for MSA in Japan, we translated the original UMSARS into Japanese in accordance with the recommendation by the International Society for Pharmacoeconomics and Outcomes Research task force for translation and cultural adaptation (Wild et al. 2005). We have then verified the reliability and validity of this Japanese version of UMSARS (Chikada et al. 2021).

7 Ubiquinol as a Drug for MSA

We conducted a pilot study of CoQ10 supplementation in one patient from a multiplex family with MSA carrying biallelic variants of *COQ2* (UMIN000010712) (Mitsui et al. 2017). Daily administration of 1200 mg of ubiquinol (the reduced form of CoQ10) substantially increased total coenzyme Q10 levels in the cerebrospinal fluid as well as in the plasma. The patient was at the advanced stage of MSA, and the various scores of clinical rating scales remained the same over 3 years. Moreover, the cerebral metabolic rate of oxygen measured by $^{15}O_2$-positron emission tomography (PET) increased by approximately 30% after administration of ubiquinol, suggesting that ubiquinol supplementation can improve mitochondrial oxidative metabolism in the brain of this patient (Mitsui et al. 2017).

We then conducted a single-center, randomized, double-blind, placebo-controlled phase 1 trial of ubiquinol in healthy male volunteers (UMIN000016695). Participants were randomly assigned to orally receive 900, 1200, or 1500 mg of ubiquinol or placebo daily for 4 weeks. Safety was assessed by examining the frequency and severity of adverse events in the ubiquinol group compared with those in the placebo group. The levels of ubiquinol in plasma and the levels of CoQ10 in leukocytes and cerebrospinal fluid were investigated (Mitsui et al. 2022). With confirmation of its safety, we subsequently started a multicenter, randomized, double-blind, placebo-controlled trial of ubiquinol in patients with MSA (UMIN000031771). Patients were randomly assigned at a 1:1 ratio to orally receive 1500 mg of ubiquinol or placebo daily for 48 weeks. The primary efficacy was the change in UMSARS part 2 score from baseline at 48 weeks. UMSARS part 2 is a motor function evaluation score evaluated by neurologists (UMSARS part 2; range 0–56, with higher scores indicating more severe symptoms). A restricted maximum-likelihood-based

repeated-measures approach is employed to evaluate the change in UMSARS part 2 score, and the least-squares means at 48 weeks are compared between groups. Whether ubiquinol supplementation is effective only for a specific group of patients (e.g., carriers of *COQ2* variants) or for the entire patient population will also be tested by a subgroup analysis.

8 Conclusion

In recent years, much attention and effort have been focused on the development of disease-modifying therapies based on the elucidated molecular pathogenesis of neurodegenerative diseases. We are hoping that the clinical trials of ubiquinol for MSA will provide a breakthrough treatment for MSA.

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State of the Art and History of Therapeutics in Ataxias

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Abstract Research into novel therapeutics suggests that treatment of cerebellar ataxia may be attainable in the future. Recently, omaveloxolone, an NRF2 activator, has become the first treatment approved by the United States Food and Drug Administration (FDA) for Friedreich's ataxia (FRDA). We performed a systematic review of clinical trials to better understand the challenges hindering the development of successful therapies, and to identify potential shortcomings in the clinical pipeline. Clinical trials published in English were identified in several worldwide scientific databases. Trials were prospective, either single- or double-blinded (including blinded video assessments for neuromodulation trials), with a change in the severity of ataxia symptoms as the primary measure. Eighty-nine controlled clinical trials were accepted for extraction, including 3625 patients. The most common therapeutic modality over the past 50 years was pharmaceutical (idebenone). SCA3 had the highest number of patients in clinical trials in spinocerebellar ataxia (SCA). Only 9% of clinical trials reported race/ethnicity, which was predominantly white in all ataxias combined (approximately 81%). The majority of clinical trials in FRDA were performed in North America and Europe, while for SCAs, most were

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5_29

performed in the USA, Europe, Japan, Taiwan, Brazil, and Cuba. Clinical trials in cerebellar ataxia reported significantly more funding sources in the last 20 years than from 1980 to 1999 ($p = 0.016$). Future ataxia research should be more inclusive and diverse while focusing on novel therapeutics.

Keywords Cerebellar ataxia · Friedreich's ataxia · Spinocerebellar · Gender · Clinical trials

1 Introduction

The cerebellum controls balance and motor coordination, and impairment of these functions due to disease or injury creates a symptom complex known as "ataxia." The term "cerebellar ataxias" refers to a group of disorders causing this constellation of symptoms as a result of damage to the cerebellum. Cerebellar dysfunction may also cause tremor, dysarthria, oculomotor abnormalities, and affective and cognitive disorders (Gellersen et al. 2017). The major causes of cerebellar ataxia include genetic mutations, neurodegeneration, autoimmunity, neoplasm, inflammation, vitamin deficiencies, structural lesions, demyelination, and stroke. In recent years, the spinocerebellar ataxias (SCAs) and Friedreich's ataxia (FRDA; the most common autosomal recessive ataxia) have been among the most studied disorders (Subramony 2017). Although omaveloxolone was recently approved by the FDA for FRDA, there are currently no other FDA-approved therapies to treat cerebellar motor dysfunction. While several therapies have been studied in clinical trials for the past 40 years, there is as yet no consensus regarding their effectiveness.

We performed a systematic review of clinical trials in this patient population to better understand the challenges hindering the development of successful therapies, and to facilitate the design of future clinical trials. We sought to understand the scope of clinical research in cerebellar ataxia spanning the last 50 years, including patient characteristics (with a particular focus on racial/ethnic diversity), the therapies evaluated, the locations in which these trials were performed, and the funding mechanisms that supported them. This endeavor was undertaken to identify potential shortcomings in the system feeding the clinical pipeline for the ataxias, as well as the opportunities for improvement in future studies.

2 Methods

We reviewed therapeutic clinical trials focused on cerebellar ataxia published between January 1970 and December 2021. Articles were compiled following searches in PubMed, Scopus, the Cochrane Central Register of Controlled Trials,

ClinicalTrials.gov, and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP). Search terms included: cerebellar ataxia, ataxia, blinded, clinical trial, and randomized. Articles needed to be written in English as a primary language. We included prospective clinical trials, either single-blinded or double-blinded (including blinded video assessments for neuromodulation trials) with randomization and a placebo arm, or a comparison of two agents for medication trials whose primary objective was improvement of ataxia. Neuromodulation trials were not required to be randomized, sham, or placebo-controlled for inclusion, but needed to be prospective and blinded, at least for rating scores. Author affiliations (primary author) were also recorded (university, hospital, etc.). The abstracts of all articles identified by the search were manually reviewed for relevance, and selected articles were additionally reviewed for relevance by reviewing the full-length text of each article. Data extraction was then performed, including: the number of patients in the trial, race/ethnicity, gender, therapeutic modality, funding source, location of research, and year of study publication. Additionally, articles were classified as "published controlled clinical trials" if they appeared in PubMed, Scopus, and Cochrane databases. If they additionally were included in "ClinicalTrials.gov" or the ICTRP without being published as a peer-reviewed article, they were included in "controlled clinical trials," rather than published controlled clinical trials (Appendix 1). The funding sources for clinical trials were tabulated and given 1 point for each source reported. The *p*-values were calculated using Wilcoxon rank-sum test or Chi-squared test, as appropriate. Statistically significant findings were evidenced with a *p*-value smaller than the alpha level at 0.05.

3 Results

Eighty-nine published controlled clinical trials in cerebellar ataxia were accepted for data extraction based on the inclusion criteria, which yielded a total of 3625 patients (Appendix 2). There were 69 additional clinical trials that met inclusion criteria that were listed in ClinicalTrials.gov or the ICTRP, but which were not subsequently published. The mean reported age was 40.26 years (standard deviation [SD] 14.565; median 40.17) in 78 of 89 published trials; 72 trials indicated gender, which was 51.92% male. Only 8 trials reported information on race/ethnicity, which was 81.42% white, 14.82% Hispanic, and 2.92% African.

There were 24 trials accepted for FRDA extraction, with 1040 patients. The mean (SD) age was 25.24 years (7.903) in the 22 trials that provided this information (median 26.05 years), with 49.54% males. Four trials recorded race/ethnicity, which was 97.06% white. For SCA, in the 15 of 16 trials that reported age, the mean age was 45 years (SD 6.276; median 44.70). Analysis of the 14 of 16 trials that reported gender revealed 49.7% of participants were male.

Twenty cerebellar ataxia published trials (22%) were performed in the United States (USA), followed by Germany (14 trials, 15.4%), Italy (13 trials, 14.3%), Japan (8 trials, 8.8%), and "other" (Fig. 1a). Published clinical ataxia trials were

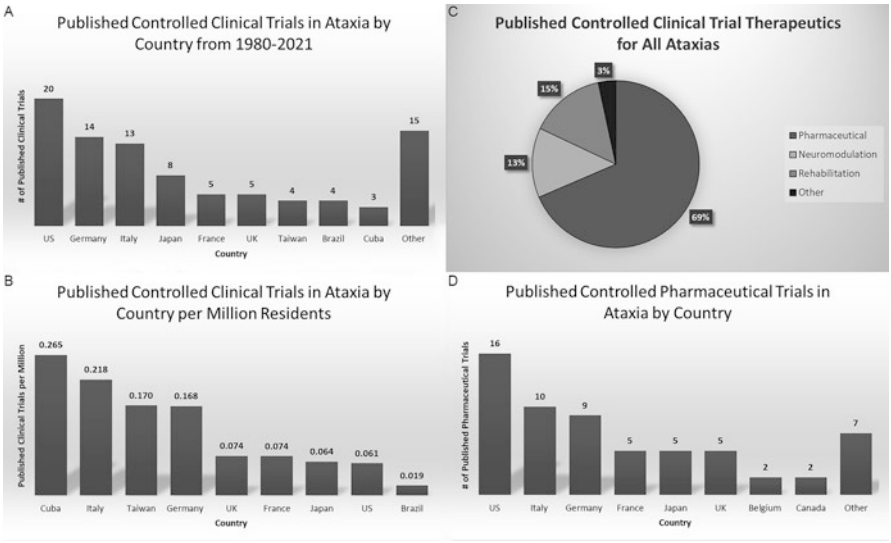


Fig. 1 (**a**) Eighty-nine published controlled clinical trials in all ataxias from 1980 to 2021 were included. Of note, two trials were completed by more than one country. "Other" includes: Belgium (2), Canada (2), China (2), Australia (2), Turkey (2), Austria, Mexico, Nigeria, Scotland, and Denmark. (**b**) Controlled clinical trials in all ataxias from 1980 to 2021 were represented per one million residents. Population data were used from 2020. (**c**) Neuromodulation includes: transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and deep brain stimulation (DBS). "Other" includes: whole body vibration and electrical muscle stimulation. (**d**) Sixty-one published controlled pharmaceutical trials from 1980 to 2021 were included. "Other" includes: Cuba, China, Brazil, Austria, Nigeria, Scotland, and Denmark

recalculated using "number of trials per million residents" using 2020 population data from the World Bank for Cuba, Italy, Germany, Japan, Brazil, and the USA (https://data.worldbank.org/indicator/SP.POP.TOTL), and CEIC Data for Taiwan (https://www.ceicdata.com/en/indicator/taiwan/population) (Fig. 1b). Using this calculation, Cuba, Italy, Taiwan, Germany, the UK, France, Japan, and the USA published the most clinical trials in cerebellar ataxia (Fig. 1b). Of the 89 published clinical trials in cerebellar ataxia of all types, 61 were pharmaceutical trials (68.5%), 12 (13.4%) were neuromodulation trials, 13 (14.6%) were rehabilitation trials, and 3 trials (3.3%) were "other" (Fig. 1c). The USA published the most pharmaceutical trials (16), followed by Italy (10), Germany (9), France (5), Japan (5), the UK (5), and "other" (Fig. 1d). The most common pharmaceutical substance evaluated in all cerebellar ataxias as a group was idebenone (Table 1).

Clinical research in ataxias was further divided into FRDA and SCAs. There were 24 published clinical trials that focused on FRDA alone, and 20 trials of "mixed ataxia," of which FRDA was included. There were additionally 20 FRDA non-published clinical trials listed in ClinicalTrials.gov and ICTRP. The USA performed the most FRDA published clinical trials (14), followed by Italy (10), Germany (7), the UK (4), France (3), and "other" (7) (Fig. 2a). When adjusting for

Table 1 List of pharmaceuticals used in published controlled clinical trials from 1980 to 2021 in all ataxias, Friedreich's ataxia (FRDA), and spinocerebellar ataxia (SCA)

Pharmaceutical	# of published clinical trials
All ataxias	
Idebenone	7
Choline chloride	4
Buspirone	3
Erythropoietin	3
L-hydroxytryptophan	3
Amantadine	2
Lecithin	2
Memantine	2
Omaveloxolone	2
Riluzole	2
Lithium	2
Physotigmine	2
L-carnitine	2
Aminopyridine	2
TMP-SMX	2
Oral zinc sulfate	1
A0001	1
BCAAs	1
CoQ(10)/vitamin E	1
Deferiprone	1
Docosahexaenoic acid	1
EPI-743	1
Interferon-γ 1b	1
Epigallocatechin gallate	1
Ondansetron	1
Rovatirelin	1
RT001	1
Thiamine hydrochloride	1
Thyrotropin-releasing hormone	1
Acetyl-DL-leucine	1
Valproic acid	1
Varenicline	1
Vigabatrin	1
Betamethasone	1
D-cylcoserine	1
Luvadaxistat	1
FRDA	
Idebenone	7
Erythropoietin	3

(continued)

Table 1 (continued)

Pharmaceutical	# of published clinical trials
L-hydroxytryptophan	3
Lecithin	2
Omaveloxolone	2
L-carnitine/creatine	2
Riluzole	2
Amantadine	2
Choline chloride	2
AC001	1
CoQ(10)/vitamin E	1
Deferiprone	1
EPI-743	1
Interferon-γ 1b	1
RT001	1
Luvadaxistat	1
Ondansetron	1
Buspirone	1
Vigabatrin	1
Physotigmine	1
Acetyl-DL-leucine	1
SCA	
Lithium	2
TMP-SMX	2
Riluzole	2
Zinc	1
Varenicline	1
Docosahexaenoic acid	1
Valproic acid	1
L-acetylcarnitine	1
Rovatirelin	1
Buspirone	1
Physotigmine	1
Branched-chain amino acid	1
Ondansetron	1
Choline chloride	1
D-cycloserine	1
Acetyl-DL-leucine	1

the number of trials per million residents, Italy led with the most clinical trials (Fig. 2b), followed by Germany, France, the UK, and the USA (Fig. 2b). Australia also conducted several trials, published and unpublished. For FRDA published controlled trials, pharmaceuticals accounted for 84% (37), 9% neuromodulation (4), and 7% rehabilitation (3) (Fig. 2c). The most common pharmaceutical evaluated for FRDA was idebenone (7 trials), followed by erythropoietin (3), *L*-hydroxytryptophan (3), and others (Table 1).

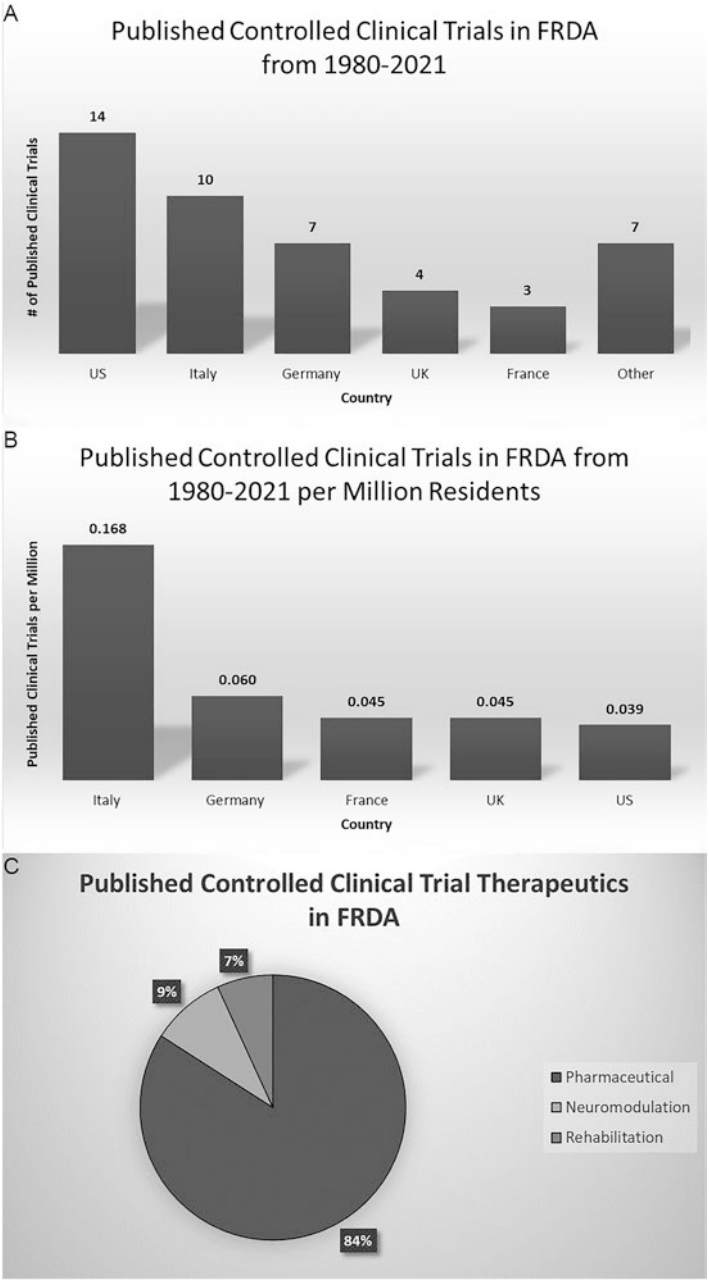


Fig. 2 (**a**) Forty-four published controlled clinical trials in Friedreich's ataxia (FRDA) from 1980 to 2021 were included. Of note, one trial was completed by more than one country. "Other" includes: Belgium (2), Canada (2), Australia, Austria, and Scotland. (**b**) Published clinical trials in FRDA from 1980 to 2021 were adjusted per million residents. Population data were used from 2020. (**c**) Therapeutics used in the 44 published controlled clinical trials that met inclusion criteria

There were 16 published clinical trials that focused on the SCAs, and 24 mixed ataxia trials that included SCA patients. Italy performed the most clinical trials (8), followed by Japan (7), Germany (5), the USA and Taiwan (4 each), Brazil and Cuba (3 each), and "other" (6) (Fig. 3a). Adjusting for population size (number of trials per million resident), Cuba performed the most clinical trials in SCA per million residents, followed by Taiwan, Italy, Germany, Japan, Brazil, and the USA (Fig. 3b). Forty-seven percent trials in SCA were pharmaceutical studies (19), followed by 25% neuromodulation (10), 20% rehabilitation (8), and 8% other (3) (Fig. 3c). The most common pharmaceutical substances studied in SCA published controlled trials were lithium (2), trimethoprim-sulfamethoxazole (TMP-SMX) (2), and riluzole (2), followed by others (Table 1). SCA3 had the highest number of patients in clinical trials (199), followed by SCA2 (123). The rest of all SCAs combined included 58 patients (SCAs 6, 7, 38, 1, 8, and 14).

There were significantly more clinical trials that reported funding in the last 20 years for cerebellar ataxia research than in the years spanning from 1980 to 1999 ($p = 0.0156$) (Fig. 4a). This also held true for SCA ($p = 0.01$) (Fig. 4c). FRDA had a trend for more funding than SCA from 1980 to 1999 ($p = 0.0827$; p-value obtained using Wilcoxon rank-sum test) (Fig. 4b). Thirty-four percent of all published cerebellar ataxia clinical trials reported government funding (36), 21% private foundation (23), 19% industry (20 trials), 19% not reported (20), and 7% private hospital/university (8) (Fig. 5a). "Government funding" was made up of the National Institute of Health (NIH) and federally funded hospitals, universities, and foundations. For published FRDA trials, government funding accounted for 31% of trials (17), 24% private foundation (13), 28% industry (15), 13% not reported (7), and 4% private hospital/university (2) (Fig. 5b). Published SCA trials reported 36% government funding (16), 22% private foundation (10), 7% industry (3), 24% not reported (11), and 11% private hospital/university (5) (Fig. 5c).

For FRDA, the government agencies that funded the most clinical trials were the NIH, the German Research Foundation, the Italian Agency for Pharmaceutics, and the Murdoch Children's Research Institute, Australia (also private funding for the latter). The most active private foundations were the Friedreich's Ataxia Research Alliance (FARA) and Ataxia UK, while active industries included Santhera Pharmaceuticals, Takeda Pharmaceuticals, Retrotope Inc., Reata Pharmaceuticals, Minoryx, and Larimar therapeutics. For SCA, government agencies that funded the most clinical trials were NIH, Hospital de Clinicas de Porto Alegre (Brazil), Cuban Ministry of Public Health, and Ministry of Science and Technology, Taiwan; the top private foundations included Deutsche Heredo-Ataxia Gesellschaft, the National Ataxia Foundation (NAF), and the Bobby Allison Research Foundation. The industry that funded SCA research was Biohaven Pharmaceuticals, and private universities/hospitals were Chang Gung Medical Research Program in Taiwan and Columbia University, New York City, USA.

Using a series of analyses, we sought to evaluate associations between various variables extracted. Among the 89 clinic trials included in this review, 52 (58%) trials investigated one specific disease (e.g., FRDA, MSA, or SCA) among which 30 (58%) of the studies reported significant findings. The other 37 (42%) trials looked at multiple diseases simultaneously (e.g., FRDA and SCA), 27 (73%) of which reported a significant improvement in primary or secondary outcomes. A

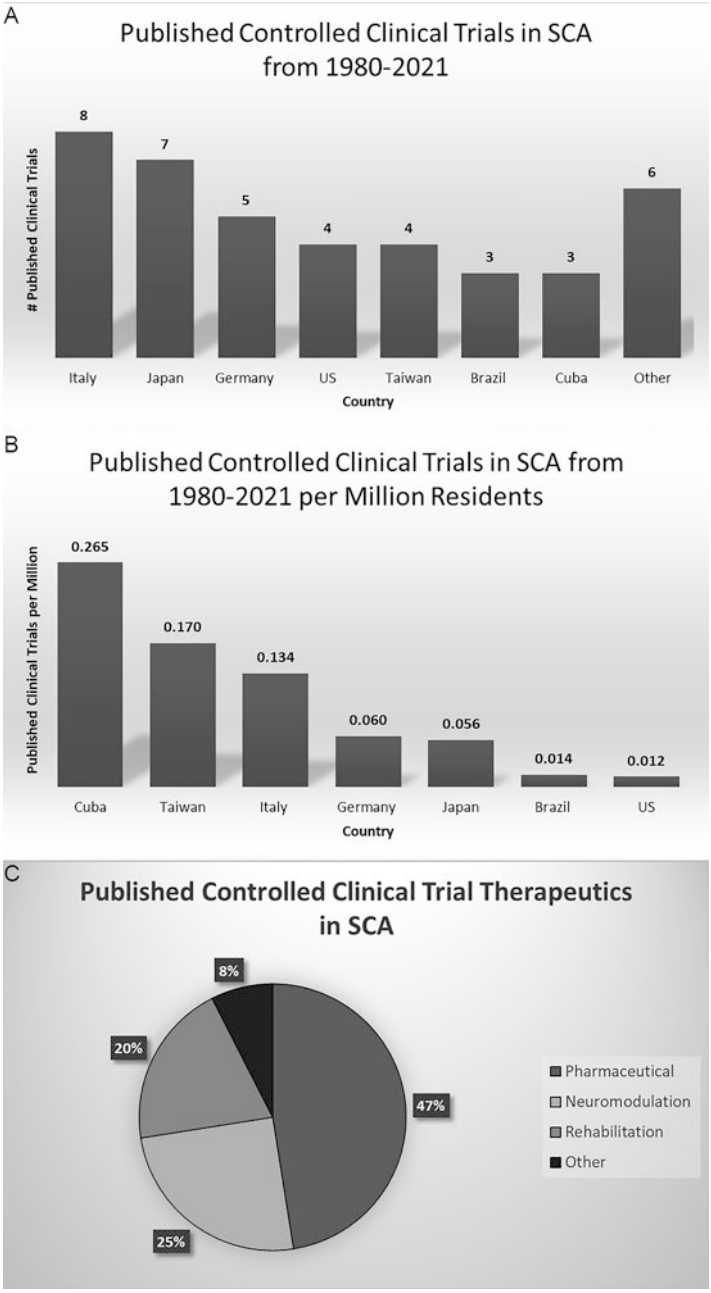


Fig. 3 (**a**) Forty published controlled clinical trials in spinocerebellar ataxia (SCA) from 1980 to 2021 were included. "Other" includes: Turkey (2), UK, Belgium, China, and Mexico. (**b**) Published clinical trials in SCA from 1980 to 2021 were adjusted per million residents. Population data were used from 2020. (**c**) Therapeutics used in the 44 published controlled clinical trials that met inclusion criteria. Neuromodulation includes: transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and deep brain stimulation (DBS). "Other" includes: whole body vibration and electrical muscle stimulation

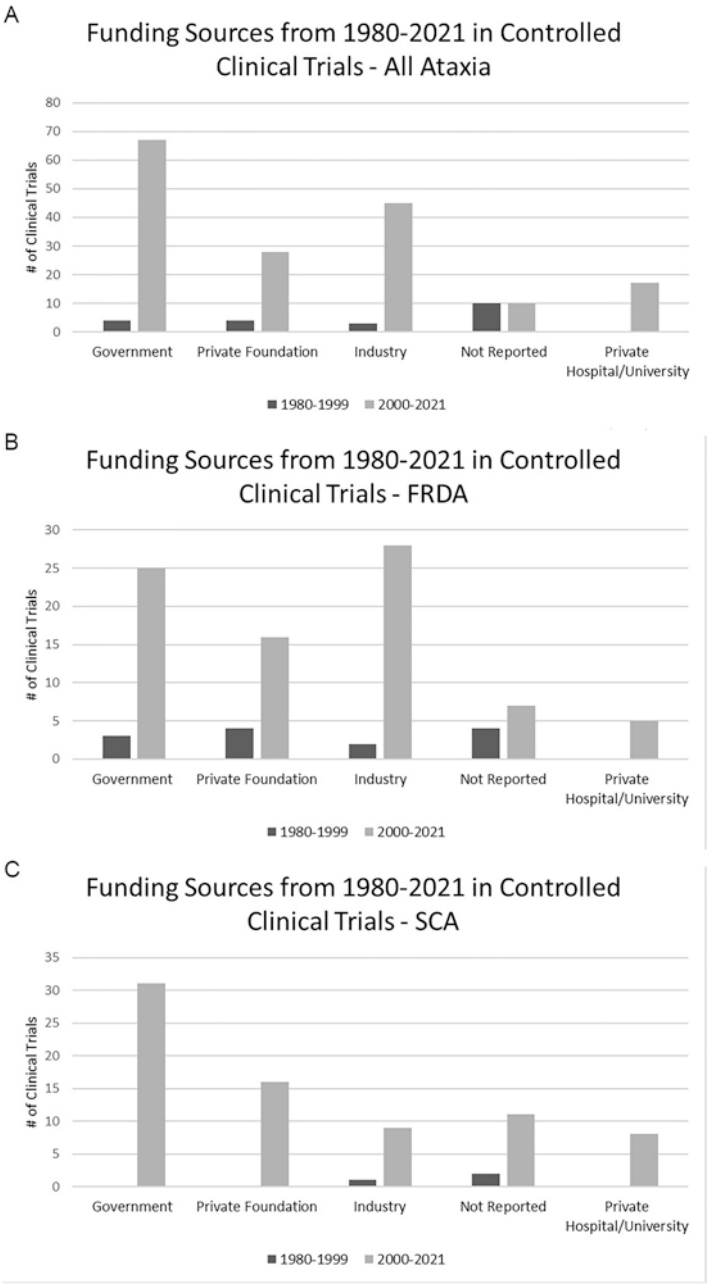


Fig. 4 (**a**) Funding sources in published controlled clinical trials in all cerebellar ataxias from 1980 to 2021 were grouped from 1980 to 1999 and 2000 to 2021. (**b**) Funding sources in published controlled Friedreich's ataxia (FRDA) clinical trials from 1980 to 2021 were grouped from 1980 to 1999 and 2000 to 2021. (**c**) Funding sources in published controlled spinocerebellar ataxia (SCA) clinical trials from 1980 to 2021 were grouped from 1980 to 1999 and 2000 to 2021

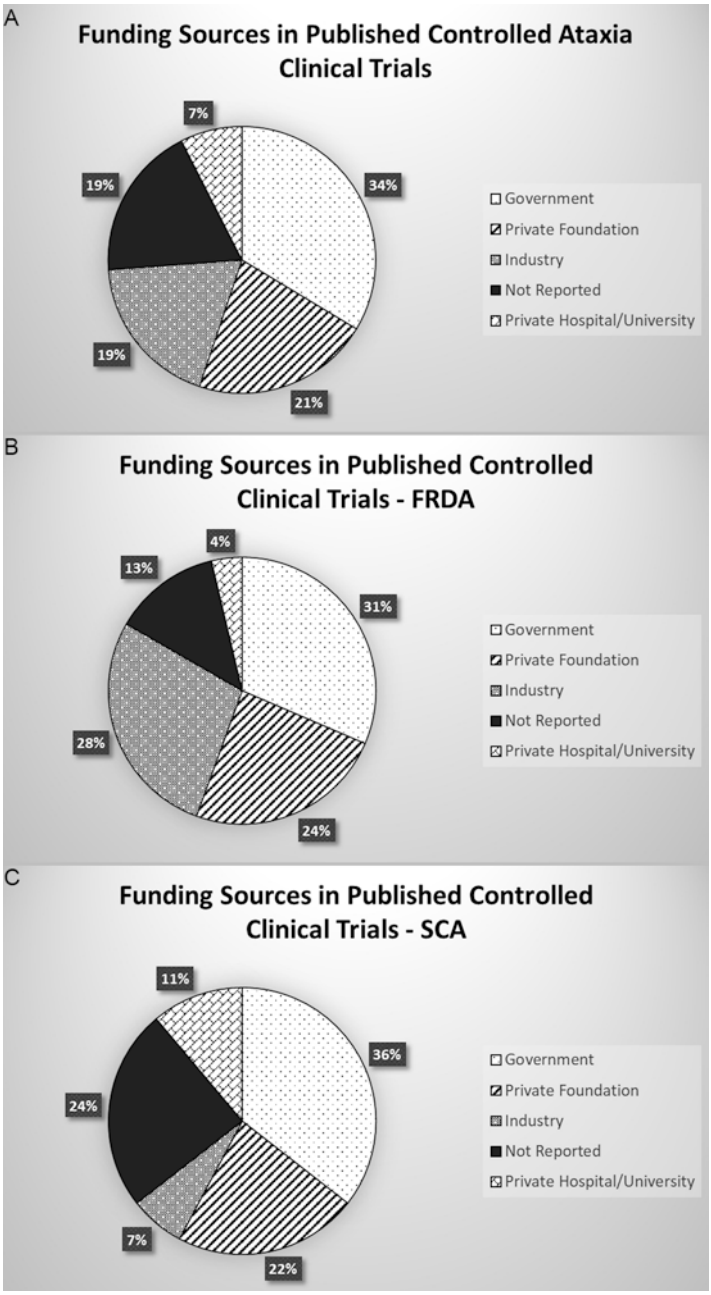


Fig. 5 (**a**) Funding sources in published controlled clinical trials in all ataxias from 1980 to 2021 were reported. Government includes: National Institute of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS), and federally funded hospitals, universities, and foundations. (**b**) Funding sources in published controlled clinical trials in Friedreich's ataxia (FRDA) from 1980 to 2021 were reported. (**c**) Funding sources in published controlled clinical trials in spinocerebellar ataxia (SCA) from 1980 to 2021 were reported

Chi-squared test showed that there was no statistically significant association between whether the study investigated only one type or multiple types of diseases and whether the study reported significant findings ($p = 0.2089$). Further, trial sample size did not differ significantly between those that reported (median = 20) and those that did not report (median = 32) significant findings ($p = 0.0729$). Taken together, among the 89 trials included in this review, neither study sample size nor whether the study investigated multiple diseases was determinant factor that affected the trials' ability of detecting a significant improvement, and the smaller-sized trials, with sufficient effect size, showed adequate power as compared with larger-sized trials.

4 Discussion

This systematic review was performed in order to better understand the past and present landscape of clinical trials in cerebellar ataxia, and to identify potential shortcomings in the clinical trial pipeline. To this end, we examined demographics such as age, race/ethnicity and gender, locations, type of therapeutics, and funding sources and trends.

The mean age of study participants in published clinical trials was 40.26 years for all types of cerebellar ataxia. The mean age in FRDA trials was approximately 25 years, while the average age of FRDA symptom onset in the community ranges between 10 and 15 years, although some sources report the range between 5 and 15 years (Cook and Giunti 2017; Indelicato et al. 2020). The average age of FRDA patients in clinical trials in this review was higher than the mean age of onset in the majority of FRDA patients in the community, suggesting that patients who enrolled in clinical trials had a longer burden of disease, or may have had more adult-onset disease. It could be argued that this disparity might have affected study outcomes, as treatment in earlier disease or younger patients might have yielded different results. However, enrollment of children in clinical trials is often considered to be challenging due to a variety of factors, including the need for parental consent, possible risk from interventions, and difficulties inherent in children participating in long study evaluations such as clinical rating scales (Joseph et al. 2015). Dedicated pediatric clinical trials in FRDA are an important undertaking moving forward in order to determine the potential efficacy of therapeutics in this population. In terms of SCAs, the average age of onset ranges from 20s to 50s, and the mean age of onset in clinical trials was similar.

Only 9% of published controlled clinical trials reported the racial/ethnic breakdown in clinical trials. In these trials, FRDA trials were overwhelmingly white, while SCA2 trials included patients of Hispanic ancestry (Cook and Giunti 2017; Schöls et al. 2004). FRDA is prevalent in Europe (particularly in the southern areas), the Middle East, India, North America, Australia, and New Zealand (Cook and Giunti 2017; Delatycki et al. 1999). However, published clinical trials in FRDA were most often performed in North America and Europe. Likewise, SCAs are

common in various locations around the world; for example, SCA3 is prevalent in Portugal, Brazil, China, Germany, and Japan (Schöls et al. 2004). SCA7 may be found in South Africa and Mexico, SCA10 is more prevalent in South and Central America, and SCA12 is found in India (Schöls et al. 2004; van Prooije et al. 2021). However, the majority of clinical trials in ataxia were performed in the USA, Europe, Japan, Taiwan, Brazil, and Cuba, with few to no trials performed in the African continent, Central America, or India. SCA2 is most prevalent in Cuba, which may enable their clinical sites to recruit large SCA2 populations with similar ethnic backgrounds. The paucity of clinical trials in some regions of the world may be a missed opportunity to understand potential genetic variability in ataxia. It is vitally important to reach out to underserved areas as there is known underrepresentation of disadvantaged people in medical research, including clinical trials. Race/ethnicity should be reported in all clinical trials, with greater efforts toward diversity (Gan et al. 2020).

The majority of controlled clinical trials in cerebellar ataxia were performed on either FRDA or SCA patients. In this review, approximately 33% of patients in clinical trials pertained to FRDA and 27% to SCA, but only 6% of research focused on MSA, 4% on FXTAS, and 0.44% on Ataxia Telangiectasia. Likewise, while there are more than 40 types of SCA, the overwhelming majority of research was performed on SCA3 and SCA2 patients, which are among the most common forms of ataxia (Schöls et al. 2004). Many of the trials in FRDA and SCAs did not focus solely on these diseases, but included additional "mixed" forms of ataxia. Several of these studies may have had inadequate power to detect efficacy, although this issue occurs in rare diseases in which recruitment of patients is challenging.

The most common therapeutic modality in ataxia over the past 50 years was "pharmaceutical," and the most frequent agent studied was idebenone for FRDA. Other treatment modalities included neuromodulation and rehabilitation, although they were less commonly performed than pharmaceutical studies. Of the studies included, only 64% demonstrated a significant improvement in ataxic outcomes. As research advances in ataxia, more clinical trials should include novel therapeutics, such as antisense oligonucleotides, gene therapy, and mitochondrial-focused therapies in FRDA, in addition to rehabilitation and neuromodulation trials.

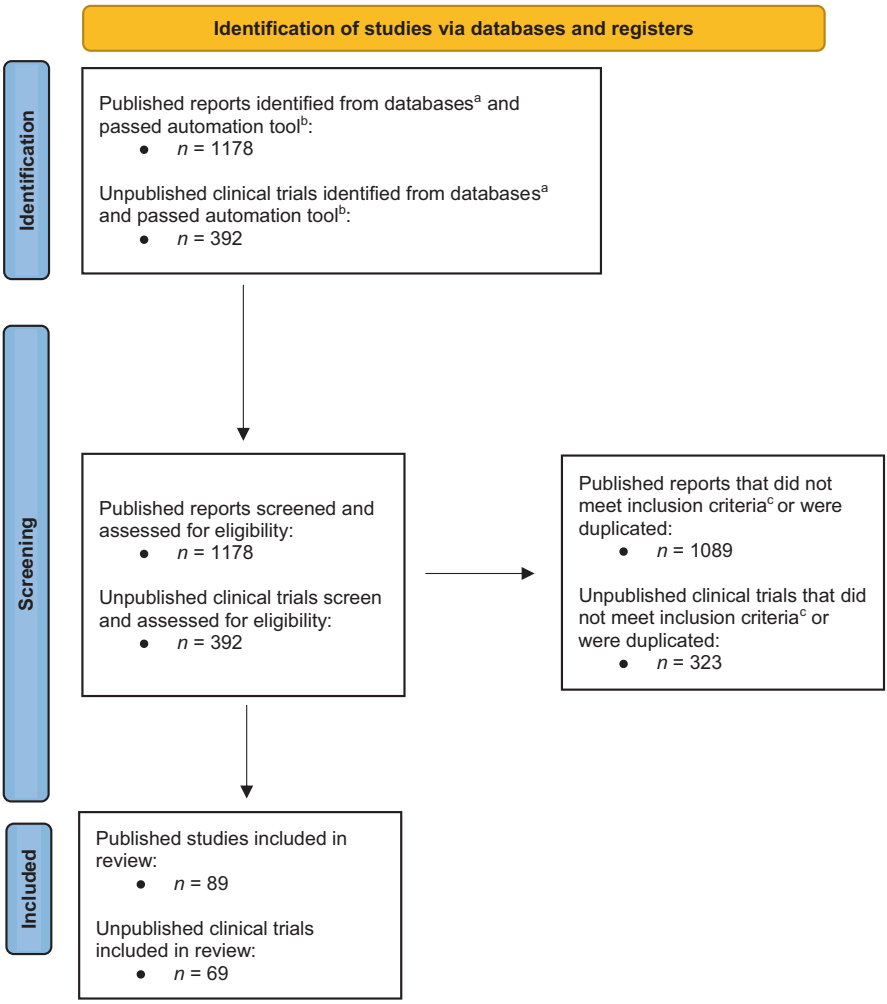
The FDA has recently granted its first approval of a drug (Omaveloxolone) for the treatment of FRDA after the publication of the study supporting a therapeutic effect of the novel Nrf2 activator (Lynch et al. 2021). FRDA pathophysiology is mediated by dysfunctional mitochondria activity, as a result of impaired Nrf2 signaling (Lynch et al. 2023). Omaveloxolone is a potent activator of the Nrf2 pathway, thus restoring mitochondria function and increasing adenosine triphosphate production. Increases in funding have produced a dramatic increase in the number of therapeutic clinical trials over the last two decades, particularly in FRDA. This is very encouraging and greatly increases the possibility of garnering sufficient evidence of a successful therapy to win FDA approval. One of the major barriers to successful drug development is the so called "Valley of Death" between laboratory discovery and execution of the very expensive Phase IIb and Phase III studies typically funded by industry. This gap is due to the lack of funding for the initial Phase I and small

Phase IIa studies essential to developing enough evidence of efficacy to convince larger pharmaceutical companies to undertake and fund the larger more expensive IIb and III studies, and also underwrite the expensive FDA application process needed for drug approval. The increase in clinical trial activity in the ataxias in our analysis was driven primarily by government and foundation funding sources, much of which was directed at earlier, smaller clinical trials, with this end in mind. Such a trajectory emphasizes the progress that can be facilitated by the concerted efforts of private foundations and organizations. In FRDA, as one example, it is clear that the FARA has been instrumental in helping to drive this increase in recent years, particularly in the USA, though many other organizations worldwide have made major contributions to this important surge. These organizations, most often driven by patients and their families, should be rightly proud of the work they have done, and should take heart that their efforts have changed the landscape of clinical research in the ataxias. We anticipate that the development and approval of a successful drug, at least for FRDA, is no longer a distant prospect, and may be imminent. We encourage our partners to continue their valiant efforts in support of the treatment and ultimate eradication of the ataxias.

Funding This study did not receive funding.

Appendix 1

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only.



From: Page et al. (2021)

[a]Databases for published papers include PubMed, Scopus, and Cochrane. Databases for unpublished clinical trials include clinicaltrials.gov and the International Clinical Trials Registry Platform (ICTRP) by the World Health Organization (WHO).

[b]Automation tool was utilized to include clinical trials in English.

[c]General inclusion criteria: improvement in ataxia, prospective clinical trials, single or double blinded (includes blinded video assessments). Inclusion criteria for medication trials include: improvement in ataxia, prospective clinical trials, single or double blinded, and placebo arm or comparison of two agents. Inclusion criteria for neuromodulation trials include: prospective clinical trials and blinded assessments. For more information, visit: http://www.prisma-statement.org/

Appendix 2

Authors	Year	Title	Therapeutic	Dx	# of Subjects	Age (mean/ range)	Outcomes assessed	Biomarker type	Improvement	*p*-Value (confidence interval)	Treatment category	Country	Gender (Males; Females)	Race?
Sehested et al.	1980	Oral choline in cerebellar ataxia	Choline chloride	6 cerebellar ataxia	6	NR	Walking, finger to nose, heel to knee	n/a	No	None	Medication	Denmark	NR	NR
Lawrence et al.	1980	The use of choline chloride in ataxic disorders	Choline chloride	3 mixed ataxia, 8 ICD, 1 familial SCD, 1 FRDA, 1 alcoholic cerebellar degeneration	14	56.5/10–76	Walking, washing, feeding, nystagmus, dysarthria, spiral drawing, tapping, handwriting, pegboard, postural stability	n/a	No	None	Medication	UK	10;4	NR
Pentland et al.	1981	Lecithin treatment in Friedreich's ataxia	Lecithin	12 FRDA	12	18.8/11–30	Ataxia rating scale	n/a	No	None	Medication	Scotland	NR	NR
Livingstone et al.	1981	Choline chloride in the treatment of cerebellar and spinocerebellar ataxia	Choline chloride	7 FRDA, 7 SCA, 6 ICA	20 (7 placebo, 7 six week dose, 6 twelve week dose)	FA (29.7), SCA (29.9), ICA (53.8)	Ataxia score	n/a	No	None	Medication	UK	10;10	NR
Kark et al.	1981	Double-blind, triple-crossover trial of low doses of oral physostigmine in inherited ataxias	Physotigmine	10 FRDA, 3 olivopontocerebellar atrophy, 2 peroneal atrophy with ataxia, 1 combined atrophy of the cerebellar atrophy, 1 AT, 1 adolescent-onset arylsulfatase deficiency, 1 Ramsay-Hunt syndrome, 1 ragged red ataxia, 1 sensory neuropathy w/ cerebellar atrophy	21	NR	Eyes, mouth, larynx, diaphragm, upper limbs, trunk, lower limbs	n/a	Yes	0.025	Medication	USA	NR	NR

Melancon et al.	1982	Oral lecithin and linoleic acid in Friedreich's ataxia: II. Clinical results	Lecithin	22 FRDA	32	NR	Muscle strength and coordination	n/a	No	None	Medication	Canada	NR	NR
Sobue et al.	1983	Controlled trial of thyrotropin releasing hormone tartrate in ataxia of spinocerebellar degenerations	Thyrotropin-releasing hormone	80 LCCA, 140 OPCA, 19 cerebellospinal form, 17 others	256 (91 control, 85 treatment 1, 80 treatment 2)	NR	Global improvement rating, ataxia improvement rating – standing, gait, speech, hand writing, tapping test	n/a	No	None	Medication	Japan	Control (62;29); treatment 1 (48;37); treatment 2 (52;28)	NR
Austin et al.	1984	Low dose choline chloride in cerebellar degeneration	Choline Chloride	5 SCD, 11 cerebellar degeneration	16	51.13/16–72	Standard ataxia assessment; handwriting, spiral copying, rapid dotting, and straight line drawing tests	n/a	No	None	Medication	UK	8;8	NR
Bonnet et al.	1986	A controlled study of oral vigabatrin (gamma-vinyl GABA) in patients with cerebellar ataxia	Vigabatrin	9 FRDA, 5 OPCA	14	30.9/16–55	Functional staging, Disability, Tapping test, walking, standing	n/a	No	None	Medication	France	9;5	NR
Trouillas et al.	1988	Improvement of cerebellar ataxia with levorotatory form of 5-hydroxytryptophan. A double-blind study with quantified data processing	L-5-hydroxytryptophan	12 CCA, 2 FRDA, 2 brain stem infarctions with cerebellar ataxia, 6 multiple sclerosis with cerebellar ataxia, 8 postsurgery ataxia	30	NR/6–80	Ataxia rating scale	n/a	Yes	0.03	Medication	France	15;15	NR
Filla et al.	1993	A double-blind cross-over trial of amantadine hydrochloride in Friedreich's ataxia	Amantadine	12 FRDA, 2 ADCA	14	27.4/13–50	FASS	n/a	No	None	Medication	Italy	6;8	NR
Adamolekun et al.	1994	A double-blind, placebo-controlled study of the efficacy of thiamine hydrochloride in a seasonal ataxia in Nigerians	Thiamine hydrochloride	14 SAS	14 (6 control, 8 treatment)	41.2/22–55: control (41.5/NA); treatment (41/NA)	Composite severity grading scale; tremor and ataxia ratings	n/a	Yes	<0.0001	Medication	Nigeria	Control (1;5); treatment (1;7)	100% African

Authors	Year	Title	Therapeutic	Dx	# of Subjects	Age (mean/ range)	Outcomes assessed	Biomarker type	Improvement	*p*-Value (confidence interval)	Treatment category	Country	Gender (Males; Females)	Race?
Wessel et al.	1995	Double-blind crossover study with levorotatory form of hydroxytryptophan in patients with degenerative cerebellar diseases	L-hydroxytry ptophan	19 FRDA, 13 CA, 7 OPCA	39	NR	Ataxia rating scale	n/a	No	None	Medication	Germany	NR	NR
Trouillas et al.	1995	Levorotatory form of 5-hydroxytryptophan in Friedreich's ataxia. Results of a double-blind drug-placebo cooperative study	L-5-hydroxytry ptophan	19 FRDA	19	25.9	Ataxia rating scale	n/a	Yes	Kinetic – 0.03	Medication	France	NR	NR
Sakai et al.	1995	Sulfamethoxazole-trimethoprim double-blind, placebo-controlled, crossover trial in Machado-Joseph disease: sulfamethoxazole-trimethoprim increases cerebrospinal fluid level of biopterin	Sulfameth oxazole-trimethoprim	8 SCA3	8	45.9/31–60	Timed testing	CSF	Yes	<0.05	Medication	Japan	7;1	NR
Trouillas et al.	1996	Treatment of cerebellar ataxia with buspirone: a double-blind study	Buspirone	19 CCA	19	49.3	Ataxia rating scale	n/a	Yes	Kinetic – 0.04, intensity of body sway – 0.009, quality of standing with feet together – 0.01	Medication	France	NR	NR
Botez et al.	1996	Amantadine hydrochloride treatment in heredodegenerative ataxias: a double blind study	Amantadine	27 FRDA, 30 OPCA	FA (27), OPCA (30)	FA (31/19–47); OPCA (40/21–62)	Ataxia rating, simple reaction time, movement time	n/a	Yes	FRDA – <0.05; olivopontocerebellar atrophies – <0.001	Medication	Canada	FA (14;13); OPCA (11;19)	NR

Trouillas et al.	1997	Buspirone, a serotonergic 5-HT1A agonist, is active in cerebellar ataxia. A new fact in favor of the serotonergic theory of ataxia	Buspirone	19 CCA	19 (9 control, 10 treatment)	Overall (49.3/NA); control (50.6/NA); treatment (48.2/NA)	Ataxia scale	n/a	Yes	Kinetic – 0.04	Medication	France	NR	NR
Wessel et al.	1997	Double-blind crossover study with physostigmine in patients with degenerative cerebellar diseases	Physostigmine	8 ICA, 7 ADCA, 2 SCA1, 2 SCA3	19	46.8	Ataxia Clinical Rating Scale	n/a	No	None	Medication	Germany	8;11	NR
Sorbi et al.	2000	Double-blind, crossover, placebo-controlled clinical trial with L-acetylcarnitine in patients with degenerative cerebellar ataxia	L-acetylcarnitine	11 FRDA, 10 ILOCA, 1 SCA1, 2 SCA2	24	36.9/18–63	Ataxia rating scale	n/a	Yes	Coordination – <0.05 at 6 months, peripheral signs in ILOCA group – <0.0006 at 3 months and <0.01 after 6 months, muscle tone in FA at 6 months – <0.0007	Medication	Italy	NA	NR
Schulte et al.	2001	Double-blind crossover trial of trimethoprim-sulfamethoxazole in spinocerebellar ataxia type 3/Machado-Joseph disease	Trimethoprim-sulfameth oxazole	22 SCA3	22 (11 control, 11 treatment)	44.7/30–69	Ataxia rating scale	n/a	No	None	Medication	Germany	12;10	NR
Schöls et al.	2001	Idebenone in patients with Friedreich ataxia	Idebenone	9 FRDA	9	34/19–54	ICARS	Echo and P-MRS	Yes	P-MRS – 0.003	Medication	Germany	4;5	NR
Shiga et al.	2002	Transcranial magnetic stimulation alleviates truncal ataxia in spinocerebellar degeneration	TMS	74 SCD	74 (35 control, 39 treatment)	Control (58.83/38–76); treatment (56.31/27–76)	Walking tests	n/a	yes	10 m walk time – <0.05; tandem stems- <0.05; standing capacity – <0.01	Other	Japan	Control (25;11); treatment (19;20)	NR

Authors	Year	Title	Therapeutic	Dx	# of Subjects	Age (mean/ range)	Outcomes assessed	Biomarker type	Improvement	*p*-Value (confidence interval)	Treatment category	Country	Gender (Males; Females)	Race?
Mori et al.	2002	Double-blind crossover study of branched-chain amino acid therapy in patients with spinocerebellar degeneration	Branched-chain amino acid	8 SCA6, 1 SCA7, 5 OPCA, 2 CCA	16	61.9/46–83	ICARS	n/a	Yes	Low dose group – <0.01; high-dose group – <0.05	Medication	Japan	6;10	NR
Ogawa et al.	2002	D-cycloserine for the treatment of ataxia in spinocerebellar degeneration	D-Cycloserine	10 MSA-C, 2 CCA, 2 SCA6, 1 SCD	15	57.9/41–74	ICARS and timed tests	n/a	Yes	Posture and gait – <0.05, total ICARS – <0.05, walking – <0.05	Medication	Japan	9;6	NR
Mariotti et al.	2003	Idebenone treatment in Friedreich patients: one-year-long randomized placebo-controlled trial	Idebenone	29 FRDA	29 (15 control, 14 treatment)	Control (26.3/20–31); treatment (26.1/22–29)	ICARS	Echo	Yes – Echo	Echo: Intraventricular septal thickness – 0.007, left ventricular posterior wall – 0.03, left ventricular mass – 0.007	Medication	Italy	Control (12;3); treatment (11;3)	NR
Bier et al.	2003	Effects of the oral form of ondansetron on cerebellar dysfunction. A multi-center double-blind study	Ondansetron	15 CCA, 7 MSA, 4 FRDA, 6 SCA, other 13	46	NR	ICARS	n/a	Yes	finger to nose decomposition – 0.049, heel to knee – 0.003, body sway eyes closed – 0.017, body sway eyes open – 0.014	Medication	Belgium	NR	NR
Schöls et al.	2005	L-carnitine and creatine in Friedreich's ataxia. A randomized, placebo-controlled crossover trial	L-carnitine/ creatine	16 FRDA	16 (6 control, 5 creatine, 5 L-carnitine)	30.7/18–55	ICARS and pegboard test	ATP production, echo, LVM	No	None	Medication	Germany	5;11	NR

Ihara et al.	2005	Influence of repetitive transcranial magnetic stimulation on disease severity and oxidative stress markers in the cerebrospinal fluid of patients with spinocerebellar degeneration	TMS	10 OPCA, 6 CCA, 4 SCA6	20	51.8	ICARS	8-OHdG, AFR, Cu, Zn-SOD protenin, Mn-SOD protein	Yes	<0.003	Other	Japan	NR	NR
Assadi et al.	2007	Treatment of spinocerebellar ataxia with buspirone	Buspirone	6 Idiopathic, 2 SCA3, 3 SCA2, 4 FRDA, 1 SCA1, 1 SCA6, 1 SCA17, 1 DRP LA	19	40.5/14–65	ICARS	n/a	No	0.24	Medication	USA	NR	NR
Di Prospero et al.	2007	Neurological effects of high-dose idebenone in patients with Friedreich's ataxia: a randomised, placebo-controlled trial	Idebenone	47 FRDA	47	13.4	FARS and ICARS	8-OHdG	No	None	Medication	USA	24;23	NR
Cooper et al.	2008	Coenzyme Q10 and vitamin E deficiency in Friedreich's ataxia: predictor of efficacy of vitamin E and coenzyme Q10 therapy	CoQ(10)/ vitamin E	43 FRDA	43 (21 low dose, 22 high dose)	Overall (24/10–58); low (26/10–46); high (22.6/11–58)	ICARS	Echo	Yes	<0.001	Medication	UK	Low (12;9); high (6;16)	NR
Lynch et al.	2010	A phase 3, double-blind, placebo-controlled trial of idebenone in Friedreich ataxia	Idebenone	70 FRDA	70 (35 control, 35 treatment)	13.7/8–18	ICARS	n/a	No	None	Medication	USA	33;37	NR
Ristori et al.	2010	Riluzole in cerebellar ataxia: a randomized, double-blind, placebo-controlled pilot trial	Riluzole	13 Ataxia, 1 FXTAS, 2 MS, 8 FRDA, 2 SCA1, 4 SCA2, 2 SCA28, 6 MSA-C, 1 Anti-Yo, 1 Anti-GAD	40 (20 control, 20 treatment)	Control (44.1/NA); Treatment (48.9/NA)	ICARS	n/a	Yes	<0.001	Medication	Italy	Control (7;13); treatment (8;12)	NR

Authors	Year	Title	Therapeutic	Dx	# of Subjects	Age (mean/range)	Outcomes assessed	Biomarker type	Improvement	*p*-Value (confidence interval)	Treatment category	Country	Gender (Males; Females)	Race?
Drinkard et al.	2010	Exercise capacity and idebenone intervention in children and adolescents with Friedreich ataxia	Idebenone	42 FRDA	42 (11 control, 31 treatment)	Overall (13.2/NA); control (13/NA); low dose (13.8/NA); intermediate dose (13.3/NA); high dose (12.7/NA)	ICARS and FARS	Echo and VO_2 max	No	None	Medication	USA	Control (7;4); low dose (3;6); medium dose (6;7); high dose (5;4)	NR
Velázquez-Pérez et al.	2011	Oral zinc sulphate supplementation for six months in SCA2 patients: a randomized, double-blind, placebo-controlled trial	Oral zinc sulfate	36 SCA2	36 (18 control, 18 treatment)	Control (39.38/18–58); treatment (42.35/33–51)	SARA	MDA	Yes-MDA	0.007	Medication	Cuba	NR	100% Hispanic
Lagedrost et al.	2011	Idebenone in Friedreich ataxia cardiomyopathy-results from a 6-month phase III study (IONIA)	Idebenone	70 FRDA	70 (24 control, 22 low dose, 24 high dose)	Control (13.7/8–18); low dose (13.9/9–17); high dose (13.4/8–18)	ICARS	Echo	No	None	Medication	USA	NR	NR
Strupp et al.	2011	A randomized trial of 4-aminopyridine in EA2 and related familial episodic ataxias	4-Amino pyridine	10 familial episodic ataxia with nystagmus	10	40.4/13–63	Ataxia attacks	n/a	Yes	0.03 (95%)	Medication	Germany	7;3	NR
Kalla et al.	2011	Comparison of 10-mg doses of 4-aminopyridine and 3,4-diaminopyridine for the treatment of downbeat nystagmus	Aminopyridine, 3,4-diamino pyridine	Cerebellar degeneration [$n = 1$], bilateral vestibulopathy [$n = 1$], bilateral vestibulopathy and cerebellar degeneration [$n = 1$], Arnold-Chiari I malformation and cerebellar ataxia [$n = 1$], cryptogenic cerebellar ataxia [$n = 4$]	8	68/58–76	Eye movement	n/a	Yes	<0.01	Medication	Germany	2;6	NR

Miyai et al.	2012	Cerebellar ataxia rehabilitation trial in degenerative cerebellar diseases	Rehabilitation	20 SCA6, 6 SCA31, 16 ICA	42 (21 Immediate and 21 control)	Immediate (63.5/40–82); delayed (61.5/41–76)	SARA, Functional Independence Measure	n/a	Yes	<0.001	Other	Japan	Immediate (13;8); delayed (9;12)	NR
Lynch et al.	2012	A0001 in Friedreich ataxia: biochemical characterization and effects in a clinical trial	A0001	31 FRDA	31 (10 control, 21 treatment)	31	FARS	Vitamin E and CoQ	Yes	Low dose – 0.04; high dose – <0.01	Medication	USA	NA	NR
Zesiewicz et al.	2012	A randomized trial of varenicline (Chantix) for the treatment of spinocerebellar ataxia type 3	Varenicline	18 SCA3	18 (9 control, 9 treatment)	Control (53.78/NA); treatment (47.44/NA)	SARA	n/a	Yes	Gait – 0.04, stance 0.03	Medication	USA	Control (6;3); treatment (4;5)	NR
Mariotti et al.	2012	Erythropoietin in Friedreich ataxia: no effect on frataxin in a randomized controlled trial	Erythropoietin	16 FRDA	16 (5 control, 11 treatment)	Control (27.4/23–36); treatment (29/19–36)	SARA, 9HPT, SF-36	Frataxin	No	None	Medication	Italy	Control (2;3); treatment (5;6)	NR
Zannolli et al.	2012	A randomized trial of oral betamethasone to reduce ataxia symptoms in ataxia telangiectasia	Betamethasone	13 AT	13	9.7	ICARS	BMI, blood pressure, blood glucose, HbA1c, cholesterol, HDL cholesterol, potassium, sodium, calcium, phosphorus, CD4+ lymphocytes or to disease pathogenesis (i.e., alpha fetoprotein)	Yes	ICARS – 0.01 (95%)	Medication	Italy	7;6	NR
Ilg et al.	2012	Video game-based coordinative training improves ataxia in children with degenerative ataxia	Coordinative training	3 arCA, 4 FRDA, 2 ADCA, 1 AOA2	10	15.4/11–20	SARA	n/a	Yes	0.0078	Other	Germany, USA	5;5	NR

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Seritan et al.	2014	Memantine for fragile X-associated tremor/ ataxia syndrome: a randomized, double-blind, placebo-controlled trial	Memantine	93 FXTAS	93 (47 control, 46 treatment)	Control (66.3/NA); treatment (64.7/NA)	CATSYS	n/a	No	None	Medication	USA	Control (28;19); treatment (32;15)	100% white
Saute et al.	2014	A randomized, phase 2 clinical trial of lithium carbonate in Machado-Joseph disease	Lithium carbonate	62 SCA3	62 (31 control, 31 treatment)	Control (40.4/NA); treatment (40.5/NA)	NESSCA and SARA	n/a	No	NESSCA-0.22(95%), SARA-0.33(95%)	Medication	Brazil	Control (14;17); treatment (16;15)	NR
Boesch et al.	2014	Safety and tolerability of carbamylated erythropoietin in Friedreich's ataxia	Erythropoietin	36 FRDA	36 (13 control, 23 treatment)	Control (28.7/NA); treatment (27.3/NA)	SARA and FARS	Frataxin, 8-Ohdg, MDA, peroxides	No	None	Medication	Austria	Control (8;5); treatment (14;9)	NR
Yang et al.	2014	Memantine effects on verbal memory in fragile X-associated tremor/ ataxia syndrome (FXTAS): a double-blind brain potential study	Memantine	41 FXTAS	41 (20 control, 21 treatment)	Control (64.7/NA); treatment (62.1/NA)	CVLT	EEG and ERP	Yes	Cued recall – congruous and incongruous – 0.037	Medication	USA	Control (14;6); treatment (11;10)	NR
Kaut et al.	2014	A randomized pilot study of stochastic vibration therapy in spinocerebellar ataxia	Whole body vibration	7 SCA1, 1 SCA2, 11 SCA3, 13 SCA6	32 (15 control, 17 treatment)	Control (57.3/NA); treatment (61.2/NA)	SARA	n/a	No	0.143	Other	Germany	Control (10;5); treatment (10;7)	NR
Pandolfo et al.	2014	Deferiprone in Friedreich ataxia: a 6-month randomized controlled trial	Deferiprone	72 FRDA	72 (17 control, 21 low dose, 20 med dose, 14 high dose)	Control (18.4); low (18.2); medium (18.5); high (21.1)	FARS and ICARS	Echo	No	FARS – 0.69, ICARS – 0.50	Medication	Belgium	Control (7;10); low (9;12); medium (9;11); high (10;4)	NR
Romano et al.	2015	Riluzole in patients with hereditary cerebellar ataxia: a randomised, double-blind, placebo-controlled trial	Riluzole	40 SCA, 20 FRDA	60 (30 control, 30 treatment)	Control (43.1/NA); treatment (46.2/NA)	SARA	ECG	Yes	0.002	Medication	Italy	Control (14;16); treatment (17;13)	NR

Saccà et al.	2015	A randomized controlled pilot trial of lithium in spinocerebellar ataxia type 2	Lithium	17 SCA2	17 (8 control, 9 treatment)	Control (38.9/NA); treatment (40.6/NA)	SARA	MRI	No	0.85	Medication	Italy	Control (5;3); treatment (4;5)	NR
Benussi et al.	2015	Cerebellar transcranial direct current stimulation in patients with ataxia: a double-blind, randomized, sham-controlled study	tDCS	5 SCA2, 1 SCA1, 2 SCA38, 1 FRDA, 1 AOA2, 6 MSA-C, 1 FXTAS, 2 sporadic adult-onset ataxia	19	53.8	SARA and ICARS	n/a	Yes	SARA – <0.001; ICARS – <0.001	Other	Germany	8;11	NR
Chang et al.	2015	Cycling regimen induces spinal circuitry plasticity and improves leg muscle coordination in individuals with spinocerebellar ataxia	Rehabilitation	20 SCA	20 (40 subjects, with 20 SCA and 20 control. This one is kinda weird)	48.9/20–65	ICARS	Electromy ography	Yes	ICARS – 0.046	Other	Taiwan	8;12	NR
Chen et al.	2015	Neuromuscular electrical stimulation of the median nerve facilitates low motor cortex excitability in patients with spinocerebellar ataxia	Neuromuscular electric stimulation	7 SCA3, 2 sporadic SCA	27 (9 young, 9 age, 9 SCA)	Controls (young-24.3), (age-41.3); SCA (43.7)	n/a	Resting motor threshold, silent period, motor evoked potential (MEP)	Yes	MEP – <0.05	Other	Taiwan	Controls (young – 4;5), (age matched – 3;6); SCA (5;4)	NR
Huang et al.	2015	Restoration of central programmed movement pattern by temporal electrical stimulation-assisted training in patients with spinal cerebellar atrophy	TMS	10 SCA3, 2 SCA6, 8 unidentified SCAs	20 (10 control, 10 treatment)	Control (51/NA); treatment (47/NA)	Goal-directed movement test	Electrom yography	Yes	0.0249	Other	Taiwan	Control (5;5); treatment (2;8)	NR
Saccà et al.	2016	Long-term effect of epoetin alfa on clinical and biochemical markers in Friedreich ataxia	Epoetin alfa	56 FRDA	56 (28 control, 28 treatment)	Overall (35.8/NA); control (36.2/NA); treatment (35.4/NA)	SARA	Frataxin	No	None	Medication	Italy	Overall (29/27); control (17;11); treatment (12;16)	NR

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Lei et al.	2016	Safety and efficacy of valproic acid treatment in SCA3/MJD patients	Valproic acid	36 SCA3	36 (12 control, 12 low dose, 12 high dose)	Control (39.5/NA); low dose (36.5/NA); high dose (33.9/NA)	SARA	n/a	Yes	0.021	Medication	China	NR	NR
Benussi et al.	2017	Long term clinical and neurophysiological effects of cerebellar transcranial direct current stimulation in patients with neurodegenerative ataxia	tDCS	5 SCA2, 2 SCA38, 1 SCA14, 1 FRDA, 1 AOA2, 4 MSA-C, 1 FXTAS, 5 SAOA	20 (8 control, 12 tDCS)	Control (49.8/NA); tDCS (55.2/NA)	SARA and ICARS	n/a	Yes	SARA – <0.01; ICARS – <0.01	Other	Italy	Control (3;5); treatment (7;5)	NR
Schatton et al.	2017	Individualized exergame training improves postural control in advanced degenerative spinocerebellar ataxia: a rater-blinded, intra-individually controlled trial	Rehabilitation	2 arCA, 5 FRDA, 1 AOA1, 2 AT	10	16/6–29	SARA	n/a	Yes	0.003	Other	Germany	7;3	NR
Manes et al.	2017	Docosahexaenoic acid is a beneficial replacement treatment for spinocerebellar ataxia 38	Docosa hexaenoic acid	10 SCA38	10 (5 control, 5 treatment)	48.7/NA	SARA and ICARS	PET scan	Yes	SARA – 0.008, ICARS – 0.02	Medication	Italy	4;6	NR
Rodríguez-Díaz et al.	2018	Neurorehabilitation therapy in spinocerebellar ataxia type 2: a 24-week, rater-blinded, randomized, controlled trial	Rehabilitation	38 SCA2	38 (19 control, 19 rehabilitation)	Control (38.78/20–58); rehabilitation (39.52/18–63)	SARA	n/a	Yes	0.002	Other	Cuba	Control (10;9); rehabilitation (13;6)	NR
Zesiewicz et al.	2018	Randomized, clinical trial of RT001: early signals of efficacy in Friedreich's ataxia	RT001	19 FRDA	19 (6 control, 13 treatment)	Control (NA/23–47); treatment (NA/18–48)	FARS-Neuro	VO_2 max	No	None	Medication	USA	Control (4;2); treatment (5;8)	NR

Wang et al.	2018	A randomized controlled pilot trial of game-based training in individuals with spinocerebellar ataxia type 3	Rehabilitation	9 SCA3	9 (4 control, 5 rehabilitation)	Control (NA/44–61); rehab (NA/51–60)	SARA	n/a	Yes	0.042	Other	Taiwan	Control (2;2); rehab (2;3)	NR
Milne et al.	2018	Can rehabilitation improve the health and well-being in Friedreich's ataxia: a randomized controlled trial?	Rehabilitation	19 FRDA	19 (9 control, 10 rehabilitation)	Control (35.9/NA); rehab (37.7/NA)	FARS	n/a	Yes – movement subscale	0.003 (95%)	Other	Australia	Control (6;3); rehab (2;8)	NR
Benussi et al.	2018	Cerebello-spinal tDCS in ataxia: a randomized, double-blind, sham-controlled, crossover trial	tDCS	7 SCA2, 6 MSA-C, 1 SCA38, 1 SCA14, 1 FRDA, 1 AOA2, 4 SAOA	20 (10 control, 10 treatment)	(54.6/NA)	SARA and ICARS	n/a	Yes	SARA – <0.001; ICARS – <0.001	Other	Italy	(10;10)	NR
Zesiewicz et al.	2018	Double-blind, randomized and controlled trial of EPI-743 in Friedreich's ataxia	EPI-743	63 FRDA	63 (21 control, 20 treatment 1, 22 treatment 2)	Control (29.7/NA); treatment 1 (28.7/NA); treatment 2 (29.1/NA)	FARS-Neuro	Echo	Yes	FARS-Neuro < 0.001	Medication	USA	Control (11;10); treatment 1 (10;10); treatment 2 (11;11)	100% white
Lynch et al.	2018	Safety, pharmacodynamics, and potential benefit of omaveloxolone in Friedreich ataxia	Omaveloxolone	69 FRDA	69 (17 control, 52 treatment)	Control (24.4/16–37); treatment (25.9/16–37)	mFARS	VO_2 max	Yes	<0.001 (95%)	Medication	USA	Control (7;10); treatment (25;27)	97% white
Tercero-Pérez et al.	2019	Effects of physical rehabilitation in patients with spinocerebellar ataxia type 7	Rehabilitation	18 SCA7	18	(39.94/NA)	SARA	Dityrosine, 2,4-dinitrophenyl hydrazine, lipid hydroperoxides, malondialdehyde, glutathione reductase, glutathione peroxidase	Yes	<0.05	Other	Mexico	(13;5)	NR

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Velázquez-Pérez et al.	2019	Neurorehabilitation improves the motor features in prodromal SCA2: a randomized, controlled trial	Rehabilitation	30 SCA2	30 (15 control, 15 treatment)	Control (38.64/18–53); treatment (38.33/25–43)	SARA	n/a	No	0.683	Other	Cuba	Control (5;10); treatment (3;12)	100% Hispanic
Barretto et al.	2019	Transcranial direct current stimulation in the treatment of cerebellar ataxia: a two-phase, double-blind, auto-matched, pilot study	tDCS	7 cerebellar ataxia	7	36.57/14–57	SARA	n/a	Yes – movement subscale	0.03	Other	Brazil	3;4	NR
Lynch et al.	2019	Randomized, double-blind, placebo-controlled study of interferon-γ 1b in Friedreich ataxia	Interferon-γ 1b	92 FRDA	92 (45 control, 47 treatment)	Control (16.1); treatment (16.5)	FARS and mFARS	Frataxin	No	None	Medication	USA	Control (19;26); treatment (21;26)	Control (97.8% white), treatment (91.5% white) – control (2.2% Hispanic), treatment (8.5% Hispanic)
Cook et al.	2019	Patient-reported outcomes in Friedreich's ataxia after withdrawal from idebenone	Idebenone	29 FRDA	29 (13 control, 16 treatment)	Control (38.7/15–66); treatment (35.8/20–73)	ICARS	n/a	Yes	0.0121	Medication	UK	Control (9;4); treatment (9;7)	NR
Manor et al.	2019	Repetitive transcranial magnetic stimulation in spinocerebellar ataxia: a pilot randomized controlled trial	TMS	1 SCA1, 1 SCA2, 13 SCA3, 3 SCA6, 1 SCA8, 1 SCA14	20 (10 sham, 10 treatment)	50.8/38–68	SARA	n/a	Yes	0.008	Other	USA	6;14	NR

Levin et al.	2019	Safety and efficacy of epigallocatechin gallate in multiple system atrophy (PROMESA): a randomised, double-blind, placebo-controlled trial	Epigallocatechin gallate	92 MSA	92 (45 control, 47 treatment)	Control (64/60–70); treatment (60/56–71)	UMSARS	n/a	No	None	Medication	Germany	55;37	NR
Vogel et al.	2019	Speech treatment improves dysarthria in multisystemic ataxia: a rater-blinded, controlled pilot-study in ARSACS	Speech therapy	7 ARCAS	7	38.6	Direct magnitude estimation	n/a	Yes	0.024	Other	Germany, Australia	2;5	NR
Song et al.	2020	Repetitive transcranial magnetic stimulation of the cerebellum improves ataxia and cerebello-fronto plasticity in multiple system atrophy: a randomized, double-blind, sham-controlled and TMS-EEG study	TMS	50 MSA	50 (25 control, 25 treatment)	Control (53.2/NA); treatment (53.1/NA)	SARA	n/a	Yes	<0.01	Other	China	Control (14;11); treatment (15;10)	NR
Nishizawa et al.	2020	Effect of rovatirelin in patients with cerebellar ataxia: two randomised double-blind placebo-controlled phase 3 trials	Rovatirelin	KPS1301 (165 SCA6, 72 SCA31, 132 CCA); KPS1305 (83 SCA6, 57 SCA31, 62 CCA)	KPS1301 [369 (123 control, 124 treatment 1, 122 treatment 2)]; KPS13015 [202 (101 control, 101 treatment 2)]	KPS1301 [control (62.1), treatment 1 (64.3), treatment 2 (62.7)]; KPS1305 [control (66.5), treatment 2 (63.5)]	SARA	n/a	Yes	0.03	Medication	Japan	KPS1301 [control (67;56), treatment 1 (64;60), treatment 2 (64;58)]; KPS1305 [control (49;52), treatment 2 (57;44)]	NR

Authors	Year	Title	Therapeutic	Dx	# of Subjects	Age (mean/range)	Outcomes assessed	Biomarker type	Improvement	*p*-Value (confidence interval)	Treatment category	Country	Gender (Males; Females)	Race?
Barbuto et al.	2020	Phase I randomized single-blinded controlled study investigating the potential benefit of aerobic exercise in degenerative cerebellar disease	Rehabilitation	6 MSA-C, 7 SCA, 7 idiopathic	20 (10 control, 10 treatment)	Control (46.1/NA); treatment (53.8/NA)	SARA	VO_2 max	Yes	SARA – <0.001 (95%); VO_2 – 0.026 (95%)	Other	USA	Control (7;3); treatment (6;4)	NR
França et al.	2020	Effects of cerebellar transcranial magnetic stimulation on ataxias: a randomized trial	TMS	8 MSA, 7 post lesion ataxia, 9 SCA 3	24	49	SARA and ICARS	n/a	Yes	SARA – 0.002; ICARS – 0.005	Other	Brazil	8;16	NR
Barbuto et al.	2021	Phase I single-blinded randomized controlled trial comparing balance and aerobic training in degenerative cerebellar disease	Rehabilitation	20 cerebellar degeneration	20	NR	SARA	VO_2 max	Yes	SARA – 0.03(95%); VO_2 – 0.04(95%)	Other	USA	NR	NR
Lynch et al.	2021	Safety and efficacy of omaveloxolone in Friedreich ataxia (MOXIe study)	Omaveloxolone	82 FRDA	82 (42 control, 40 treatment)	Control (23.6/NA); treatment (24.2/NA)	mFARS	Echo	Yes	0.014	Medication	USA	Control (28;14); treatment (16;24)	Placebo (95.2% white); treatment (100% white)
Benussi et al.	2021	Motor and cognitive outcomes of cerebello-spinal stimulation in neurodegenerative ataxia	tDCS	5 SCA1, 12 SCA2, 1 SCA14, 1 SCA28, 5 SCA38, 10 MSA-C, 17 SAOA, 7 FRDA, 3 cerebellar ataxia w/ neuropathy and vestibular areflexia syndrome	61 (28 sham-real, 33 real-real)	56.9	SARA and ICARS	n/a	Yes	SARA – 0.004; ICARS – <0.001	Other	Italy	27;34	NR

Wang et al.	2021	Results of a randomized double-blind study evaluating luvadaxistat in adults with Friedreich ataxia	Luvadaxistat	67 FRDA	67	Placebo (32.5), treatment – low (31.1), treatment – high (29.6)	mFARS	L- and D-serine	No	None	Medication	USA	30;37	NR
Özvar et al.	2021	Immediate effects of local vibration and whole-body vibration on postural control in patients with ataxia: an assessor-blind, cross-over randomized trial	Whole body vibration	13 SCA, 8 MS	21	39.43	Static balance	n/a	Yes	Posterior and left limits of stability – 0.05	Other	Turkey	9;12	NR
Ayvat et al.	2021	The effects of exergame on postural control in individuals with ataxia: a rater-blinded, randomized controlled, cross-over study	Rehabilitation	12 MS, 5 SCA	17 (8 group 1, 9 group 2)	Group 1 (21.5–52), group 2 (26–37)	ICARS	n/a	Yes	0.015	Other	Turkey	6;11	NR
Feil et al.	2021	Safety and efficacy of acetyl-DL-leucine in certain types of cerebellar ataxia	Acetyl-DL-Leucine	FRDA, SCA, and others (80 hereditary, 25 nonhereditary)	108	54.8	SARA	n/a	No	None	Medication	Germany	53;55	NR
Cury et al.	2021	Safety and outcomes of dentate nucleus deep brain stimulation for cerebellar ataxia	DBS	2 SCA3, 2 stroke, 1 cerebral palsy	5	46.8	SARA and FTMRS	n/a	Yes	FTMRS – 0.039	Other	Brazil	3;2	NR

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B.-w. Soong et al. (eds.), *Trials for Cerebellar Ataxias*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-031-24345-5