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Review

Emerging therapies in hereditary ataxias

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Recent investigations have defined the pathophysiological basis of many hereditary ataxias (HAs), including loss-of-function as well as gain-of-function mechanisms at either the RNA or protein level. Preclinical studies have assessed gene editing, gene and protein replacement, gene enhancement, and gene knockdown strategies. Methodologies include viral vector delivery of genes, oligonucleotide therapies, cell-penetrating peptides, synthetic transcription factors, and technologies to deliver therapies to defined targets. In this review, we focus on Friedreich ataxia (FRDA) and the polyglutamine ataxias in which translational research is active. However, much remains to be done to identify safe and effective molecules, create ideal delivery methods, and perform innovative clinical trials to prove the safety and efficacy of treatments for these rare but devastating diseases.

Highlights

Pathophysiological understanding of genetic ataxias stemming from identification of the underlying mutations has led to identification of both upstream and downstream targets for treatment aimed at neuroprotection and cellular protection.

Many novel strategies, such as gene replacement, gene editing, oligonucleotide therapeutics, protein replacement, and antibodies directed at toxic proteins, are being developed.

Numerous downstream cellular pathways are disturbed by these mutations, and it is possible that some of these may also be useful targets of effective interventions.

Choosing the best intervention and bringing it to clinical trial remains a challenge for numerous reasons, including the rare nature of many of these variable and slowly progressive diseases and lack of adequate natural history and biomarker measures.

Inherited ataxias: therapeutic possibilities

HAs are rare, progressive neurodegenerative diseases that lead to imbalance, poor coordination, dysarthria, loss of ambulation, and early mortality. Hundreds of genes are associated with ataxias with **autosomal recessive** (**AR**; see Glossary), **autosomal dominant (AD)**, X-linked, and mitochondrial inheritance [1,2] (Box 1). HAs have onset from early in life to late adulthood, and almost none have approved therapies [3,4]. Although the motor difficulties arise primarily from cerebellar pathology, many HAs have evidence for more widespread central nervous system (CNS) lesions. This needs to be considered when CNS targets for drug delivery are contemplated. In addition, some HAs have extra-CNS pathology such as cardiomyopathy. The rapid understanding of the cell biology of the ataxias beginning in the 1990s has raised the possibility of neuroprotective therapies for the HAs.

In this review, we focus on FRDA and the polyglutamine spinocerebellar ataxias (SCAs) since these are the most prevalent ataxias and a strong scientific basis has been developed for human translation. The evolving pathophysiological understanding of these diseases allows us to conceptualize both upstream therapeutic targets, which are very close to the gene defect, such as the gene itself and its related RNA and protein, and a variety of downstream disturbances triggered by the mutant gene (Figure 1). For FRDA, we summarize recent work on antioxidant and ferroptosis-related therapies followed by discussion on transcription elongation factors, direct protein and gene replacement, gene editing, and other ways to increase frataxin (FXN) levels. In the SCAs, we discuss work on gene knockdown strategies and gene editing as well as some downstream mechanisms. It should be noted that for some rare inherited ataxias, such as ataxia with vitamin E deficiency, abetalipoproteinemia, Glut-1 deficiency, Niemann-Pick type C, and cerebrotendinous xanthomatosis, traditional biochemistry-based approaches including diets and vitamin supplements have provided some benefits, but these are not discussed here.

AR ataxias

AR ataxias result from biallelic mutations and usually lead to loss of function of the gene [5]. The most common HA, FRDA, is an AR ataxia and occurs with a prevalence of 1 in 40 000 in Indo-Caucasian

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Box 1. Hereditary ataxias

Hereditary ataxias (HAs) are rare or ultrarare diseases inherited in an autosomal dominant (AD), autosomal recessive (AR), X-linked, or mitochondrial fashion.

- AD ataxias
  - Progressive ataxias (spinocerebellar ataxias, or SCAs); the number following 'SCA' indicates the genotype. There are over 40 SCAs and some diseases that are not named as SCAs but have ataxia as a prominent symptomatology.
    - Related to CAG repeat expansions in coding sequences (PolyQ ataxias): SCA 1,SCA 2, SCA 3, SCA 6, SCA7, SCA 17, DRPLA (dentatorubral pallidoluysian atrophy)
    - Related to noncoding repeat expansions: SCA 8, SCA 10, SCA 12, SCA 27b, SCA 31, SCA 36, SCA 37
    - Conventional mutations: SCA 5, SCA 11, SCA 14, SCA 15, SCA 16, SCA 19/22, SCA 21, SCA 23, SCA 25, SCA 26, SCA 27a, SCA 28, SCA 29, SCA 34, SCA 35, SCA 38, SCA 40, SCA 41
    - Copy number variation: SCA 20, SCA 39
  - Episodic ataxias (EAs): the number following EA indicates the genotype.
    - EA 1 through 8
- AR ataxias: many of these are labeled as AR ataxia because ataxia is the most prominent feature; there are also other disorders, not named as ataxias, with ataxia as part of a more complex phenotype. More than 100 genes have been identified in this category.
  - Related to repeat expansions: Friedreich ataxia, CANVAS (cerebellar ataxia neuropathy vestibular areflexia) syndrome
  - Related to DNA repair mechanisms: ataxia telangiectasia, ataxia with oculomotor apraxia types 1 and 2, others
  - Related to possible mitochondrial dysfunction: AR ataxia of Charlevoix Saguenay, POLG related, SPG 7, others
  - Other rare diseases, including many metabolic and storage diseases
- X-linked ataxias
  - Related to repeat expansion: fragile X tremor ataxia syndrome
  - Other types
- Ataxias related to mitochondrial (mt)DNA mutations: mtDNA mutations leading to many clinical syndromes such as Kearns–Sayre and mitochondrial encephalomyopathy with ragged red fibers can have ataxia as a feature.
- Friedreich ataxia, SCA1, SCA2, SCA3, and SCA6 are the most common among these. More recently discovered genotypes such as CANVAS and SCA27b may have a high prevalence, but this is not yet fully defined.

Glossary

Adeno-associated virus (AAV): best accepted viral vector for gene delivery in human trials.
Antisense oligonucleotides (ASOs): single-stranded nucleotide sequences used to target mRNA of interest.
Autosomal dominant (AD): inheritance pattern with disorder resulting from a heterozygous mutation.
Autosomal recessive (AR): inheritance pattern with disorder resulting from biallelic mutations.
Cisterna magna (CM): a large spinal fluid space around the brainstem and cerebellum.
Dorsal root ganglia (DRG): contain primary sensory neurons innervating peripheral sensory receptors.
Echocardiogram (ECHO): imaging modality for the heart.
Modified Friedreich Ataxia Rating Scale: used to quantify neurological deficits in Friedreich ataxia.
Repeat-associated non-ATG translation (RAN): novel translational mechanism found in repeat expansion associated diseases.
YG8R mouse model: a cross between an FXN transgenic mouse with GAA (human) expansion with a heterozygous FXN knockout that has a neurological phenotype and reduced FXN levels. Complete knockout of FXN is lethal, and many other models do not have a phenotype.

populations [6], although the more recently characterized CANVAS (cerebellar ataxia, neuropathy, vestibular areflexia syndrome) may also be highly prevalent [7]. FRDA has served as a model for translational approaches to the ataxias, and we summarize these efforts in some detail. Other AR ataxias may be amenable to similar approaches but face barriers such as poor understanding of pathogenic events and paucity of natural history data.

FRDA

FRDA is related to an unstable biallelic expansion of an intronic guanine-adenine-adenine (GAA) repeat in the *FXN* gene that leads to reduced transcription of the gene and deficiency of the protein FXN; rarely, a heterozygous expansion is coupled with a conventional mutation in the nonexpanded allele [8]. Most cases have onset in the late first or second decade of life, but some have onset beyond age 25. Progressive neurological deficits result from degeneration of the sensory system, the cerebellum, and the cortical motor neurons, and the associated cardiomyopathy leads to early mortality. The size of the shorter GAA repeat correlates positively with disease severity and negatively with age at onset and FXN levels [9]. FXN is a nuclear encoded mitochondrial protein that is processed to its mature form in the mitochondria and has a role in the synthesis of iron–sulfur (Fe–S) clusters, which serve as components of the electron transport chain and the Krebs cycle [10]. Reduction in Fe–S clusters resulting from FXN deficiency leads to a dysfunctional electron transport chain, abnormal iron homeostasis, oxidative stress, and impairment of antioxidant defense mechanisms.

Downstream strategies. Targeting oxidative stress: trials targeting oxidative stress and iron overload using readily available agents such as idebenone, coenzyme Q10, and deferiprone did not produce meaningful results [11,12]; low efficacy, perhaps from biodistribution characteristics,

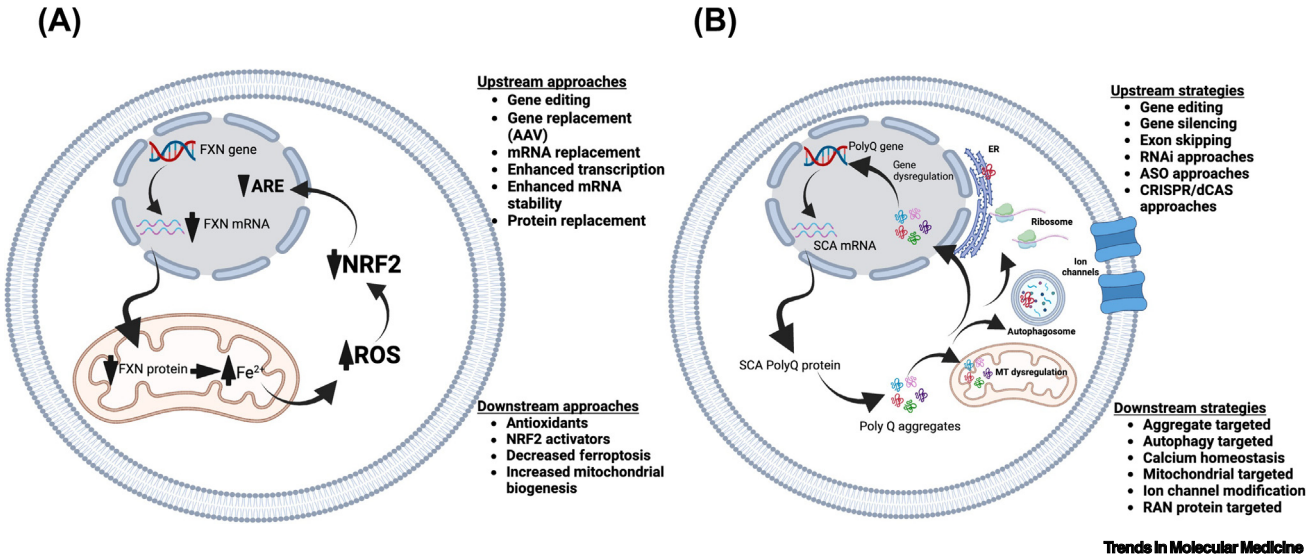

Figure 1. Key pathogenic events and therapeutic targets in Friedreich ataxia and polyglutamine spinocerebellar ataxias (SCAs). (A) In Friedreich ataxia, reduced transcription through the frataxin (FXN) gene leads to deficiency of FXN. FXN is a mitochondrial protein, and its deficiency leads to oxidative stress together with impairment of antioxidant defense mechanisms mediated by Nrf2 and antioxidant responsive elements (AREs). Upstream therapeutic strategies include direct gene therapy, agents that can enhance FXN transcription and protein replacement, whereas downstream strategies target oxidative stress and Nrf2 activation. (B) In polyglutamine spinocerebellar ataxias, the mutant allele is transcribed and translated, leading to abnormal gain of function at RNA and protein levels as well as aberrant interactions and protein aggregation. Upstream therapeutic strategies involve knockdown of the mutant allele using interfering RNA (RNAi) or antisense technologies with gene editing also as a possibility. Many downstream pathways are disturbed, such as autophagy, calcium homeostasis, mitochondrial function, abnormal ion channel function, and generation of repeat associated non-ATG (RAN) proteins. Their potential as therapeutic targets remains to be determined. This figure was created using BioRender (https://biorender.com/). Abbreviations: AAV, adeno-associated virus; ASO, antisense oligonucleotide; ER, endoplasmic reticulum; MT, mitochondria; ROS, reactive oxygen species.

prevalence of adverse effects, and trial design, may have contributed to these results. Molecules that influence various aspects of oxidative stress continue to be of interest in FRDA.

The only medication for FRDA approved officially by the FDA and the European Medicines Agency (EMA) is omaveloxolone, which has recently been reviewed [13]. Nrf2 is a transcription factor that regulates the expression of antioxidant molecules. Normally, oxidative stress causes translocation of Nrf2 to the nucleus, where it drives the antioxidant response. In cellular and mouse models of FRDA, Nrf2 does not translocate in a normal fashion, and the antioxidant response is not robust [14,15]. Omaveloxolone is a triterpenoid analogue that is a potent Nrf2 activator, and preclinical studies documented rescue of mitochondrial function in cells from patients with FRDA and the CNS of mouse models [16]. In a Phase 2 double-blind study (NCT02255435[i]), it led to a significant stabilization of neurological function compared with placebo, as indicated by the **modified Friedreich Ataxia Rating Scale** score in patients over the age of 16 [17]. Many secondary outcome measures improved significantly or showed a trend. Further analyses of long-term open-label extension data as well as a 'propensity-matched analysis' comparing treated patients with a set of 'controls' from a natural history dataset have continued to support the effectiveness of omaveloxolone [18,19]. Omvaleoxolone was generally well tolerated, with elevation of liver enzymes and gastrointestinal discomfort being more common. Liver enzyme changes were mild and transient, with no other evidence for hepatotoxicity. B-type natriuretic peptide was slightly elevated with omaveloxolone, but no fluid retention occurred. Changes in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels may need monitoring.

Dimethylfumarate, an approved drug for multiple sclerosis, is known to be an Nrf2 activator. In FRDA lymphoblasts and mouse models, it increased FXN mRNA and protein in a dose-

dependent manner by potentially reducing the formation of DNA–RNA hybrid R loops [20]. There was also an increase in mitochondrial copy number. These findings have led to an early phase trial in patients with FRDA to assess FXN levels and mitochondrial function [21].

Targeting mitochondrial biogenesis: resveratrol is a natural polyphenolic compound with antioxidant and anti-inflammatory properties and can drive mitochondrial biogenesis. It has been shown to increase FXN mRNA and protein in patient-derived cells and in the brain of the **YG8R mouse model**, which has the human FXN gene with expanded GAA repeats and exhibits a significant decrease in cerebellar frataxin levels [22]. However, another study failed to document an FXN response in IPS-derived neuronal cells from patients with FRDA [23]. In an open label study using 2.5 g and 5 g per day doses, this drug did not increase FXN levels but improved neurological rating scales [24]. A micronized form of resveratrol is being studied now in an early-phase trial (NCT03933163[ii]).

Gene expression studies in FRDA have shown a downregulation of the peroxisome proliferator-activated receptor γ and its coactivator (PPARγ/PGC1α) pathway. This led to an early study in 2013 with a PPAR agonist, pioglitazone, a drug already approved for diabetes, but the results of this trial are not available (NCT00811681[iii]). More recently, a metabolite of pioglitazone, leriglitazone, was studied in a double-blind randomized fashion (NCT03917225[iv]). The primary endpoint, magnetic resonance imaging (MRI)-derived area of the cervical spinal cord, was not met, but iron levels in the deep cerebellar nuclei were greater in placebo-treated subjects, suggesting a potential treatment response [25].

Targeting ferroptosis: ferroptosis is a form of cell death triggered by iron-dependent accumulation of lipid peroxides, especially in polyunsaturated fatty acids in cell membranes, leading to cellular compromise. RT001 is a dideuterosynthetic homolog of linoleic acid in which hydrogen has been substituted with deuterium and can reduce lipid peroxidation. In a recent study, RT001 was assessed in a double-blind placebo-controlled study (NCT04102501[v]) over an 11-month period but did not improve maximum oxygen consumption during cardiopulmonary exercise testing and other secondary measures [26]. Vatiquinone is an alpha tocotrienol quinone that inhibits 15-lipoxygenase, an oxidoreductase enzyme that controls inflammation, oxidative stress, glutathione levels, and ferroptosis and is currently in trial (NCT04577352[vi]). Another mitochondrially targeted drug in clinical trial is elamipretide, a peptide that improves mitochondrial membrane stability (NCT05168774[vii]). The lack of clear efficacy of such strategies may be related to the fact that mitochondrial dysfunction is one of many downstream pathways altered in the disease.

Upstream strategies. Strategies to upregulate FXN expression involve direct gene replacement, gene editing, improving transcription through the expanded repeat, increasing mRNA or protein stability, and direct protein replacement.

Synthetic transcription elongation factors: an attractive approach in FRDA is the design of compounds that can directly upregulate transcription through the expanded GAA repeat. The GAA expansion leads to repressive histone modifications associated with unusual DNA conformations and inhibits elongation of the transcript. However, attempts to elevate FXN levels using freely diffusing histone deacetylase inhibitors failed [27]. JQ1, a thienotriazolodiazepine, is a potent inhibitor of bromodomain proteins such as BRD4 and enables RNA polymerase II to overcome barriers for productive elongation of transcripts. Taking advantage of JQ1, a synthetic transcription elongation factor (Syn-TEF1) that uses a linear polyamide to flexibly tether JQ1 to the GAA, has been developed [28]. Tethering of JQ1 to the GAA-targeting polyamide ligand in Syn-TEF1 allows the transcription elongation machinery to surmount the transcription hindrance associated

with the GAA expansion. The authors showed that this elevates FXN mRNA and protein to wild-type levels and restores aconitase function in patient-derived cells, including neurons and cardiac myocytes. Global transcription was not perturbed. A Phase 1 trial is ongoing, and patients have received 100-, 200-, or 300-mg doses intravenously over 1 week (NCT05285540[viii], NCT05573698[ix]). There was upregulation of FXN mRNA by 30% at the end of the third dose, with no major safety issues. Further experience is needed to document safety and evidence for target engagement in relevant tissues. Concern may arise from increased transcription through the expanded repeat that may lead to unwanted effects such as RNA toxic gain of function or increased **repeat-associated non-ATG (RAN)** proteins.

Protein replacement: a direct protein replacement has been developed, using a cell-penetrating peptide (transactivator of transcription, or TAT) fused to FXN in a variety of models of FRDA [29]. TAT has a short amino acid sequence, GRKKRRQRRRPQ, that allows internalization into cells and is capable of crossing the blood–brain barrier. TAT-FXN could enter and localize to mitochondria as well as be processed to the appropriate mature form; the location of the TAT sequence upstream of the mitochondrial targeting sequence allows TAT to be processed out after entry into mitochondria. TAT-FXN reduced the cytotoxicity induced by iron in FRDA fibroblasts. Conditional knockout mice treated with TAT-FXN had a longer lifespan, better weight gain, and improved cardiac function and morphology. No data were provided for any CNS rescue. In a Phase 1 clinical trial (NCT05579691[x]), repeated subcutaneous injections of the agent (CTI-1601) over a 28-day period has been tolerated well and led to a dose-dependent increase in FXN to levels greater than 33–59% of control values in skin cells, and long-term studies are being planned [30].

Gene therapy: recent approval of direct gene therapy for diseases such as spinal muscular atrophy (SMA) and Duchenne muscular dystrophy has led to optimism for a similar approach to FRDA. **Adeno-associated virus (AAV)** has become the preferred vector system for gene delivery because of its tropism for both the CNS and cardiac targets in FRDA. The availability of several serotypes further increases tropism for different tissues, and its nonintegrating nature limits genotoxicity [31,32]. The issues to be considered when planning gene therapy for FRDA include the choice of the serotype that can target both the CNS and systemic organs affected in FRDA, the various targets that need to be transduced to achieve meaningful results, delivery methods that can transduce all the relevant regions, doses to be employed, potential toxicity resulting from immune responses and overexpression, and prevalence of naturally occurring antibodies in the population that can limit efficacy. Several serotypes have been reported to have CNS tropism, but AAV9 and AAVrh10 have emerged as the most useful candidates [32]. It is certain that new serotypes will become available as research advances to optimize the vectors for specific targets [33].

The targets that need to be transduced in FRDA include the CNS, heart, skeletal muscle, and pancreas. Within the CNS, it has been suggested that transduction of deep cerebellar nuclei may be the most important and that the sensory neurons in the **dorsal root ganglia (DRG)** may not be rescuable in symptomatic subjects, but this may be debatable [34]. There is clinical and pathologic evidence for loss of cortical motor neurons, making this an important target. Other nervous system targets include the auditory and visual system, which are affected in late stages in some patients, and local delivery to the retina may rescue of visual loss. There are ongoing trials that have targeted the heart alone on the basis of a diagnosis of cardiomyopathy based on **echocardiogram** (**ECHO**) findings (NCT05445323[xi], NCT05302271[xii]). The problems with this approach include lack of precise natural history data on cardiomyopathy, exclusion of the participants from future AAV therapies that can target the CNS, and patients' perception of balance difficulties as their most important symptom [35,36].

Systemic delivery by the intravenous (IV) route can be considered for both the CNS and extra-CNS targets, but this may need large doses, leading to potential adverse events and less-than-optimal delivery to CNS targets [37]. Several methods for CNS delivery have been explored, including intrathecal (IT) injections either by lumbar puncture (LP) or into the **cisterna magna (CM)**, delivery to the CM using a microcatheter introduced in the lumbar region, intracerebroventricular (ICV) injections, and intraparenchymal injections, which mostly target local regions [38–41]. In general, IT delivery in the CM achieves better results than in the lumbar regions, with viral genome and transgene products being found in the cortex, cerebellum, and brainstem as well as in the spinal cord and DRG [39,40]. LP delivery tends to reveal a caudal to rostral gradient in regions transduced, whereas CM and ICV delivery can achieve better results in more rostral regions. There can be variability within a given region, as well as differential expression in neurons and glial cells. MRI using a contrast agent allows real-time monitoring of product diffusion within the cerebrospinal fluid (CSF) but can increase the injection volume with concerns for increased intracranial pressure [42]. Nonhuman primate (NHP) studies suggest a role for the size of the organism, and placing the organism in the head-down position after lumbar injection has had conflicting results; there is also concern for extra-CNS leakage with the LP and CM injections [41]. Doses employed for CNS delivery are smaller than for systemic injections, limiting toxicity, but the vector spills over in significant amounts to systemic organs [43]. A combined approach of simultaneous IV and CSF delivery can also limit the total dose.

Immune responses to gene therapy can lead to serious adverse events, such as cardiac, hepatic, platelet, and renal abnormalities; loss of transgene efficacy; and inability to dose individuals who have naturally occurring immunity to the viral vectors or who have had previous gene therapy [44,45].

Several studies have assessed FXN gene replacement in animal models [46–49]. These have documented improvements in cardiac and neurological function in tissue-specific knockout models of FRDA. Although treating the mice before symptom onset preserved their weight, cardiac function, and histology, treating the mice after symptom onset also led to rapid reversal of cardiac abnormalities. Studies have also noted the possibility of toxicity from overexpression [50], and strategies are needed to avoid it [51].

Box 2. RNA-targeted therapeutics for gene manipulation

Therapeutic molecules that target RNA have become a major tool in the treatment of genetic diseases. RNA-based therapeutics may use either RNAi or ASO technology to accomplish their goals.

RNAi therapy mimics natural gene regulatory mechanisms such as small interfering RNA (siRNA) or microRNA (miRNA). Natural siRNAs are double-stranded molecules transcribed from cellular genes and are processed by the nuclease Dicer located in both cytoplasm and nucleus to the siRNA duplex, typically 20–24 nt long. siRNA duplex is incorporated into the RNA induced silencing complex (RISC), where the siRNA is unwound so that a single strand remains in the RISC; this can bind to its complementary target mRNA, which is then degraded, leading to reduction in the target mRNA and corresponding protein. The silencing efficacy of any siRNA depends on the region of the target mRNA to which it is complementary. siRNAs can be designed for specific mRNA targets of interest and delivered using chemical modifications or designed as short hairpin RNA (shRNA) that can be delivered in a viral vector for long-term expression of the desired siRNA. Natural miRNAs are similar to siRNAs but have partial complementarity to mRNA and thus can target multiple mRNAs, usually at the 3′ untranslated region (UTR). They are processed by the same type of cytoplasmic processing as siRNAs in the RISC complex but are capable of translational repression as well as RNA degradation.

ASOs are short, synthetic, single-stranded RNA or DNA sequences that are complementary to an mRNA of interest. Unlike siRNA and miRNA, they can have multiple mechanisms of action. They can induce target mRNA degradation by recruiting RNase H found in both cytoplasm and nucleus, or they can act by an ‘occupation only’ mechanism that can be used to switch splicing patterns, interfere with binding of specific RNA binding proteins, or interfere with ribosomal entry and reduce translational efficiency. ASOs themselves are rapidly degraded and need chemical modifications to endow druglike properties for better stability and binding affinity and fewer adverse effects.

Table 1. Preclinical studies with RNAi in various SCAs[a]

Disease	Molecular therapy	Model	Mode of delivery	Molecular response	Phenotypic response	Refs
SCA1	Recombinant AAV serotype 1–expressed shRNAs targeting ataxin-1	B05 mice, PKJ cell–specific	Injection into medial cerebellar lobules IV and V at 7 weeks old	5–10% transduction of PKJ cells, reduced ataxin-1, resolution of NI	Better cerebellar pathology; improved motor performance	[79]
SCA1	AAV expressing inhibitory miRNA	Knockin mice (154Q)	Bilateral DCN at 5 weeks old before motor phenotype	ATXN1 mRNA reduced by 20% and protein by 58–72% in cerebellum and brainstem	Preserved rotarod response, better gait, preserved molecular layer, and reversal of transcriptional changes	[71]
SCA1	Recombinant AAV expressing miS1 RNAi	B05 mice	Bilateral DCN before motor phenotype DCN injection at 12 weeks after symptom onset	ATXN1 suppression of >50% in cerebellum Dose-dependent suppression of ATXN1 in cerebellum	Better rotarod performance at higher doses; normalization of MRS abnormalities Improved rotarod performance at 20 weeks at high doses; restoration of transcriptional alterations	[72]
SCA3	AAV expressing inhibitory miRNA	YACMJD84.2 mice (Q84/Q84 mice)	Bilateral cerebellar injection at 6 to 8 weeks old	Cerebellum well transduced, less transduction in brainstem and thalamus, suppression of ATXN3 mRNA to 54% and protein to 59%	No improvement in motor performance or survival despite sustained miRNA expression and ATXN3 depletion likely because of localized delivery	[73]
SCA3	Lentiviral vector with shRNA targeting SNP for allele-specific silencing	Rat model with striatal pathology induced by vector delivered mutant ataxin-3	Bilateral coinjection in striatum of mutant ataxin-3 and shRNA	Reduced NI number and size; preservation of neuronal markers	Not assessed	[74]
SCA3	Lentiviral vectors encoding shAtx3 (allele-specific silencing of ataxin-3)	Tg mice expressing truncated ATXN 3, Q69, PKJ cell–specific	Injection of shAtx3 into cerebellum at 3 weeks old (symptomatic)	Reduced NI and preserved cerebellar morphology	Improved gait, balance, locomotor activity, and exploratory activity; reduced anxiety in shAtx3 mice	[76]
SCA3	Lentiviral vectors encoding shRNA against human mutant ataxin-3 (allele-specific targeting)	SCA3 mice model (C57BL/6) induced by vector-delivered mutant ataxin-3 in mouse cerebellum	Coinjection of full-length mutant ataxin-3 and shRNA into cerebellum of wild-type mice	Decreased soluble mutant ataxin-3 by 52% and aggregated mutant ataxin-3 by 66%; reduced mutant ataxin-3 intranuclear aggregation	Improved cerebellar pathology; preserved balance, motor coordination, and gait; reduced hyperactivity	[82]
SCA3	AAV expressing anti-ATXN3 miRNA	SCA3 MJD84.2 mice	Injection into DCN of 2-month-old mice	ATXN3 mRNA reduced 71.2 ± 10.6%; 34% reduction of mutant ataxin-3; reduced nuclear accumulation of mutant human ataxin-3 by 80% in DCN	Not assessed	[77]
SCA3	Lentiviral vectors encoding endogenous miRNA	SCA3 mice model (C57BL/6) induced by vector-delivered mutant ataxin-3 in mouse striatum	Coinjection of lentiviral vectors encoding mutATXN3 and endoge-nous miRNA into striatum of 5-week-old mice	Transduction in striatum; reduced mutant ataxin-3 mRNA and protein expression	Not assessed	[78]
SCA3	Stable nucleic acid lipid particles encapsulating siRNA targeting mutant ataxin-3	Striatal lentiviral model and ataxin-3 [Q69] Tg mice	IV injections of RVG-9r-targeted liposomes encapsulating siMutAtax3 on 3 consecutive days	32 ± 5.2% decreased mutant ataxin-3 mRNA and 32 ± 7.1% decreased protein in cerebellum; preserved PKJ cell number; decreased mutant ataxin-3 aggregates; significantly decreased cerebellar granular layer atrophy	Significantly better on beam walking and footprint tests for balance, motor coordination, and gait	[80]

(continued on next page)

Table 1. (continued)

Disease	Molecular therapy	Model	Mode of delivery	Molecular response	Phenotypic response	Refs
SCA6	miR-3191-5p miRNA that targets CACNA1A internal ribosomal entry site (IRES)	Mice expressing AAV9-mediated CACNA1A IRES–driven α1ACTSCA6	Coinjection into right lateral ventricle of neonatal mice AAV9-α1ACT-Q33 and AAV9-Q33–miR-3191-5p	Reduced PKJ cell degeneration; miR-3191-5p inhibition of translation initiation of CACNA1A IRES–driven α1ACTSCA6	Reduced ataxia and motor deficits	[81]
SCA7	siRNA cloned into artificial miRNA expression vectors (miS4)	BAC-Prp-SCA7-92Q SCA7 mice	Subretinal injection at 7 weeks old	Significant decrease in retinal ataxin-7 mRNA and protein expression 1 month postinjection and transduction of several retinal cell types at 30 weeks old	No toxicity	[70]
SCA7	siRNA cloned into artificial miRNA expression vectors (miS4)	BAC-Prp-SCA7-92Q SCA7 mice	Bilateral injection into DCN at 7 weeks old, presymptomatic	50% reduction in ataxin-7 mRNA and 35% reduction in protein at 33 weeks; 80% reduction in NIs	Improved motor performance and gait	[75]
SCA7	Recombinant AAV expressing shRNAs targeting CAG repeats for possible allele-specific effects	SCA7 (140Q/5Q) knockin mice	Injection into right retro-orbital vein sinus at 4.5 to 5.5 weeks old	Different constructs with varying selectivity for the expanded allele and varying safety profile	Better motor performance	[83]

[a]Abbreviations: DCN, deep cerebellar nuclei; MRS, magnetic resonance spectroscopy; NI, nuclear inclusion; PKJ, Purkinje; shRNA, short hairpin RNA; Tg, transgenic.

Gene editing and antisense strategies: gene editing strategies have been assessed in preclinical studies, showing rescue of phenotype by excising the GAA expansion in cell lines from FRDA mouse models and IPS-derived cells from patients with FRDA. Given the complexity of delivering such molecules to the CNS and potential for off-target effects, a strategy of *ex vivo* editing in hematopoietic stem and progenitor cells followed by reinfusing the patients has been suggested as well [52]. Other strategies to elevate FXN expression have included **antisense oligonucleotides (ASOs)** that target the 5′ and 3′ end of the FXN mRNA to stabilize it and ASOs that target the GAA repeat to alleviate chromatin changes induced by the expansion [53,54].

Thus, strategies to upregulate FXN levels are poised to be or are already in human trials and hold promise for a truly effective treatment for this devastating disease.

AD ataxias

SCAs result from heterozygous mutations in numerous genes that are indicated by a number following the SCA designation [1]. SCAs are rare, with a combined prevalence estimated to be 2.7 in 100 000, with SCA3 being the most common, followed by SCA2 and SCA6 [55]; the recently identified SCA27b may also have high prevalence [56]. The common SCAs, such as SCAs 1, 2, 3, 6, and 7, are caused by unstable exonic expansions of repetitive cytosine, adenine, guanine (CAG) tracts coding for glutamines and are known as polyglutamine (polyQ) SCAs [57]; less common SCAs result from expansions in noncoding regions or from conventional mutations. Interestingly, in a population-based study of subjects with no ataxia, 10.7% and 1.3% of subjects had an intermediate range or pathogenic range of SCA expansion, respectively [58]. We focus on the polyQ ataxias, in which the pathogenesis is increasingly understood and clinical trials are imminent. The pathophysiology of these disorders is complex, but there is general agreement that loss of function of the gene is an unlikely factor. Although all the genotypes share a similar phenotype, the various proteins encoded by these genes have different and incompletely elucidated functions, such as transcriptional repression [59], RNA processing [60], deubiquitination, protein homeostasis [61], and transcriptional coactivation [62]. Formation of aggregates of the mutant

Table 2. Preclinical studies with ASOs in SCAs[a]

Disease	Molecular therapy	Model	Mode of delivery	Molecular response	Phenotypic response	Refs
SCA1	Non-allele-specific Atxn1 RNA targeting	Atxn1 mice 154Q/Q2	ICV bolus at 5 weeks and 8–9 weeks (pre- and post-symptom onset), single dose	mRNA decrease in brainstem greater than cerebellum, better in PKJ cells	Partial improvement in motor performance; injection at 5 weeks allowed preservation of rotarod and longer lifespan; better transcriptomics	[92]
SCA2	ASO7	Pcp2-ATXN2-Q127 mice and BAC-Q72 SCA2 mice	ICV injection, 8 weeks old at onset of motor phenotype	ASO localized to PKJ cells; reduced ATXN2 mRNA >60% and ataxin-2 protein in cerebellum	Significant motor improvement; rescue of dysregulated proteins; restored PKJ cell firing frequency	[87]
SCA3	Anti-ATXN3 ASO	Tg SCA3 mice (Q84/Q84)	ICV injection into right lateral ventricle	Reduced mutant ATXN3 mRNA and protein	Reversed motor deficits; decreased nuclear ATXN3 accumulation; increased PKJ neuron firing frequency	[84]
SCA3	Anti-ATXN3 ASO	Tg homozygous YACMJD84.2 mice (Q84 SCA3 mice)	ICV injection to right lateral ventricle every 12 weeks starting 7–12 weeks old	Decreased ATXN3 in brainstem and lesser decrease in cerebellum; reduced nuclear accumulation	ASO treated mice had improved motor activity and rescue of MRS abnormalities	[88]
SCA3	Several exon 10–targeting ASOs leading to exon skipping	Hemizygous MJD84.2 SCA3 male mice	ICV injection at 2 to 2.5 months old followed by two more ICV injections 2 and 3 weeks after initial injection	Dose-dependent protein truncation lacking the polyQ tract with significantly reduced nuclear localization of ataxin-3 in ASO-treated mice	Not assessed	[90]
SCA3	ASO-mediated gene suppression of ATXN3	Tg homozygous YACMJD84.2 (C57BL/6) mice	ICV injection into right lateral ventricle at 8 weeks old	Rescue of reduced K channel transcripts	Rescued locomotor deficits to wild-type levels; restoration of PKJ neuron excitability to wild-type levels	[91]
SCA3	Several ASOs targeting human ATXN3	YACMJD84.2 (Q84) mice and (CMV) MJD-Q135 (Q135) mice	ICV injection into right lateral ventricle at 8 weeks old	Treated mice showed reduced ATXN3 protein in several brain regions >50% and decreased neuronal nuclear ATXN3	Not assessed	[93]
SCA3 and SCA1	CAG repeat-targeting ASO	MJD84.2 hemizygous mouse model for SCA3 SCA1 (154Q/2Q) heterozygous knockin mice	ICV administration weekly for 6 weeks in 10–14-week-old SCA3 MJD84.2 mice Weekly ICV injection in 7-week-old SCA1 (154Q/2Q) mice	Reduced mutATXN3 in cerebellum, brainstem, and hippocampus in MJD84.2 mice associated with exon skipping Reduced mutATXN1 levels in cerebellum, brainstem, spinal cord, hippocampus, striatum, and thalamus in SCA1 154Q/2Q mice	Motor performance not assessed, but magnitude of mutant ataxin reduction predicted to be effective	[85]
SCA7	ASO-mediated ataxin-7 knockdown	SCA7 266Q knockin mice	Intravitreal injection of ataxin-7 ASO in 4-week-old mice	>60% less ataxin-7 RNA expression in ASO-treated eye compared with control; significant decrease in polyQ-ataxin-7 protein aggregation in ASO-treated retina	Rescue of cone photoreceptor function and retinal protein expression including after visual dysfunction onset	[86]

[a]Abbreviations: ICV, intracerebroventricular; MRS, magnetic resonance spectroscopy; PKJ, Purkinje; Tg, transgenic.

protein, including oligomers, protofibrils, fibrils, and inclusions, is a hallmark of polyQ ataxias, and these are believed to drive the cytotoxicity by aberrant interactions and sequestration of other proteins [63,64]. These data suggest that an upstream strategy involving knockdown of mutant RNA and protein should have a therapeutic benefit in polyQ SCAs (Figure 1).

Upstream strategies

Both interfering RNA (RNAi) technology and ASOs have been used in several preclinical studies to document this possibility. Box 2 summarizes the various mechanisms of RNA targeted therapies [65–69].

RNAi therapy: RNAi therapy has been reported in several *in vivo* models of SCAs 1, 3, 6, and 7 [70–83] (Table 1). Most of these studies delivered the RNAi using AAV vectors by direct injection into the cerebellum, usually the deep nuclei, and documented an array of effects, including variable knockdown of mutant RNA and protein, reduction in nuclear aggregation of toxic proteins, improvement in cerebellar pathology, reversal of downstream transcriptional changes, and improved motor performance. Some studies used rat or mouse models with localized pathology from lentivirus-delivered mutant ataxins with simultaneous delivery of the RNAi [74,76,78,82]. An IV strategy using stable nucleic acid lipid particles has been used in a rat model of SCA3 as well [80]. In SCA6, an internal ribosomal entry site coding for an alternate gene product that drives neurotoxicity was targeted for the selective inhibition of its translation by an miRNA. Most of the studies have documented only partial knockdown of the mRNA and protein and have delivered the product before symptom onset.

Antisense therapy: similarly, several *in vivo* studies have assessed ASOs as a therapy for SCAs [84–93] (Table 2). All of them used ICV injections, except for one study in SCA7 that used intravitreal injections to target retinal photoreceptors. Also, most of the ASOs induced target RNA degradation, but a strategy to skip the CAG -containing exon was used in one study [90]. Variable but partial knockdown of the target ataxins was noted in relevant tissues, such as cerebellum and brainstem, with reduction in nuclear aggregation, rescue of Purkinje cell firing abnormalities, and variable rescue of motor performance measures.

One of the concerns with these strategies is the inability to have allele-specific knockdown in most cases. Knockout of ataxins in mice usually leads to minimal phenotypes, but biochemical alterations have been seen with downregulation of ataxins [94,95]. Allele-specific targeting is possible if the mutant allele has an SNP that is in linkage disequilibrium [74], or the long CAG tract itself can be targeted [83,85,90]. Exon skipping to exclude the CAG tract and creating a truncated ataxin can be another strategy [87]. CAG targeting ASOs can potentially be applied to multiple polyQ ataxias [85].

RNA targeted molecules do not readily cross the blood–brain barrier, and their cellular uptake is less than optimal [96]. Delivery of ASOs into the CSF is reported to lead to distribution diffusely in the spinal cord and brain, but the majority of the dose appears quickly in the systemic circulation [97,98]. In the three children who died during the nusinersen trial for SMA, therapeutic levels of the ASO were found in the spinal cord and brain tissues, but there was a caudal–rostral gradient in ASO concentration. A study of the distribution of an ASO in NHPs after a single IT injection found the highest levels in the lumbar cord and motor cortex and lower levels in the brainstem and cerebellum, regions that are heavily affected in the SCAs [99]. RNAi has been delivered to animal models using viral vectors as discussed before. However, modifications, including various scaffolds, conjugation, and nanoparticle-based methods, may allow even peripheral administration for CNS entry [100]. Nusinersen, approved for SMA, has been well tolerated except for transient proteinuria and thrombocytopenia. In the study of tofersen in superoxide dismutase 1

Clinician's corner

The only currently approved medication in hereditary ataxia (HA) is omaveloxolone for molecularly confirmed patients with Friedreich ataxia older than 16 years.

Clinicians have an opportunity to precisely characterize most patients with ataxia at a genetic level, and this is very important for research participation and ending the diagnostic odyssey.

Patients and families should be educated regarding many ongoing natural history and biomarker studies worldwide and the global translational research collaborations being developed, such as the Friedreich Ataxia Global Clinical Consortium and the Ataxia Global Initiative. These organizations welcome direct contact from patients, families, and caregivers worldwide.

Patients should be educated regarding various support groups as well as websites such as ClinicalTrials.gov, where they can find valuable information.

(SOD1) amyotrophic lateral sclerosis (ALS), however, serious adverse events occurred in 7%, including myelitis, aseptic meningitis, increased intracranial pressure, and papilledema, and many had CSF pleocytosis [101]. Other human studies of ASOs in different disorders have been discontinued for various reasons: C9Orf72 ALS (lack of efficacy signals), Huntington disease (lack of efficacy signals and worsening with more frequent dosing), and SCA3 (nonclinical safety data).

Gene editing: gene editing using CAG repeat targeting nucleases is another possibility. Preclinical work suggests that careful selection of guide strands allows targeted deletion of the CAG tracts with little off-target effect [102]. Newer strategies such as the use of nickases may improve specificity. Delivery of gene editing therapies to the CNS will also be challenging.

Downstream strategies

Numerous downstream pathways have been noted to be disrupted in SCAs and may provide therapeutic targets, but the effectiveness of these remains to be assessed. Translation leading to RAN proteins has been shown to drive toxicity in several diseases related to repeat expansions [103]. RAN proteins seem to induce neuroinflammation, disrupt the immune system, and induce neurodegeneration. Immunotherapy and metformin, which can inhibit RAN translation, have been suggested as therapeutic possibilities [103,104]. Abnormal calcium signaling may also be a possible target; mutations in many genes that code for calcium signaling proteins lead to ataxia, and abnormal calcium signaling has been found in some polyQ ataxias [105]. Preclinical studies suggest some effect of dantrolene, a calcium release inhibitor, in SCA2 and SCA3 models [106]. A role for ion channels in cerebellar disease is suggested by mutations in potassium channels that cause ataxia as well as reduced ion channel transcripts and abnormal Purkinje cell firing in polyQ ataxias [107,108], and restoring ion channel function may alleviate neurodegeneration. Finally, autophagy has been noted to be abnormal in SCA3 models, and pharmacologic and genetic manipulation of autophagy has had some effect on SCA3 models [109].

Concluding remarks

Over a short period of time, research has unraveled significant pathogenic pathways in the common HAs, bringing us to the threshold of effective neuroprotective therapies. Challenges remain in identifying safe and effective molecules ready for human trials, developing efficient delivery systems, avoiding off-target effects, and predicting long-term safety. These include identifying ideal viral and non-viral vectors for therapeutic delivery to the CNS, improving immune tolerance to viral vectors to lessen adverse events, and allowing repeated dosing if necessary, improving our ability for allele-specific silencing of mutant genes, allowing better delivery of nucleotide therapies to the nervous system, and preventing off-target effects (see Outstanding questions); active research in these areas is likely to overcome many of these challenges. The promise of gene editing remains to be fulfilled in the translational arena. Translational research is hampered by many issues related to the rare and variable nature of the ataxias [110] and paucity of sensitive outcome measures and biomarkers (see Clinician's corner). In addition, increasing dialogue between patient advocacy groups, researchers, and regulatory agencies may overcome some of the impediments to approval of such therapies. Thus, we strongly believe that the field of neuroprotective therapy in the HAs is at the threshold of a very promising future.

Outstanding questions

Can we collaborate and optimize gene editing and replacement strategies for such CNS disorders in the context of rapidly developing information about viral and non-viral vectors and a competitive environment?

What needs to be done to regulate transgene expression to avoid overexpression?

Can we overcome the challenges of immune responses to currently used viral vectors in order to minimize adverse events, improve transduction efficiency, and allow dosing of individuals with preexisting immunity or redose previously dosed individuals?

What developments are needed to deliver oligonucleotide therapies more easily and effectively to the nervous system?

How can we achieve allele-specific silencing more consistently? How can we be sure knockdown of both mutant and wild-type alleles will have no long-term consequences?

How can we perform clinical trials in such rare diseases more efficiently? How do we identify qualified biomarkers that may allow speedier trials with smaller sample sizes?

How can we develop clinically meaningful measures of function, especially in early-stage disease, that can satisfy regulatory agencies?

What will be the implications of treating presymptomatic individuals once an effective treatment is identified? What will be needed to perform clinical trials in such individuals?

How can we extend these efforts to the rarer ataxias? Can N-of-1 trials and designer therapeutics for rare gene mutations be more feasible in the future?

How do we integrate bench researchers, clinical investigators, regulatory agencies, and patient communities better to achieve these goals?

What will be the financial implications of such novel therapies?

Declaration of interests

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Resources

[i]https://classic.clinicaltrials.gov/ct2/show/NCT02255435

[ii]https://classic.clinicaltrials.gov/ct2/show/NCT03933163

[iii]https://classic.clinicaltrials.gov/ct2/show/NCT00811681

[iv]https://classic.clinicaltrials.gov/ct2/show/NCT03917225

[v]https://classic.clinicaltrials.gov/ProvidedDocs/01/NCT04102501/Prot_SAP_000.pdf

[vi]https://classic.clinicaltrials.gov/ct2/show/NCT04577352

[vii]https://classic.clinicaltrials.gov/ct2/history/NCT05168774

[viii]https://classic.clinicaltrials.gov/ct2/show/NCT05285540

[ix]https://clinicaltrials.gov/study/NCT05573698

[x]https://classic.clinicaltrials.gov/ct2/history/NCT05579691

[xi]https://clinicaltrials.gov/study/NCT05445323

[xii]https://classic.clinicaltrials.gov/ct2/show/NCT05302271

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