Chapter 18

Repeat expansion disorders*

Sharan R. Srinivasan[1,2], Claudio Melo de Gusmao[1,2,3], Joanna A. Korecka[1,2] and Vikram Khurana[1,2]

[1]Division of Movement Disorders, Department of Neurology, Brigham and Women's Hospital, Boston, MA, United States; [2]Department of Neurology, Harvard Medical School, Boston, MA, United States; [3]Department of Neurology, Boston Children's Hospital, Boston, MA, United States

Chapter outline

Introduction

There are thousands of repetitive regions in the human genome. Nucleotide sequences that occur repeatedly, known as tandem repeats, may be composed of dinucleotides, trinucleotides, or more (polynucleotide repeats). While many are evolutionarily conserved, about 2000 of these tandem repeats are specific to humans. These sequences are divided into subgroups according to their size into satellite, minisatellite, and microsatellite DNA. Microsatellites in noncoding DNA have been used predominantly in forensic, paternity, and linkage studies, while repeat expansions in or near genes can drive specific human diseases. Most commonly, symptoms from pathological repeat expansions involve the nervous system. Despite decades of research, it remains unclear why repeat expansions selectively perturb the nervous system, despite ubiquitous distribution in human tissues of altered gene products.

The repeat expansion disorders (REDs) comprise a highly diverse set of diseases including spinocerebellar ataxias (SCAs), Dentatorubral-Pallidoluysian Atrophy (DRPLA), Friedreich's Ataxia (FRDA), Fragile X Syndrome (FXS) and Fragile X Tremor-Ataxia syndrome (FXTAS), Spinobulbar muscular atrophy (SBMA, also known as Kennedy's Disease), myotonic dystrophies, oculopharyngeal muscular dystrophy (OPMD),

* Thoughts on teaching about the neurobiology of diseases section available at the beginning of the book and Index terms are available at the end of the book.

Neurobiology of Brain Disorders. https://doi.org/10.1016/B978-0-323-85654-6.00048-4

Unverricht–Lundborg myoclonic epilepsy (EPM1), C9ORF72 Frontotemporal dementia–Amyotrophic lateral sclerosis (FTD-ALS), Fuchs endothelial corneal dystrophy (FECD), and Huntington's Disease (HD).

REDs are difficult to diagnose precisely. First, each RED can be associated with a multitude of clinical presentations. Second, each particular presentation (for example, cerebellar ataxia) can in turn be caused by a multitude of genetic lesions (Table 18.1). These diseases are all progressive and disabling, if not fatal. There is not a single well-established preventative or disease-modifying medication for any of them, and no robust symptomatic treatment exists for commonly represented phenotypes, such as cerebellar ataxia.

In this chapter, we aim to explore the molecular, clinical, and pathophysiological aspects of repeat expansion disorders with a focus on diseases that cause neurodegenerative movement disorders. Following a brief clinical description of select disorders, we will detail disease mechanisms with the view to highlight pathophysiological similarities and differences. Our mechanistic exploration from the microscopic to macroscopic begins with the fundamentals of genetic expansion and the specific nucleotide-level properties that drive genetic risk for these diseases. Next, we explore areas of subcellular dysfunction that drive pathophysiology. Expanding outward, we will discuss specific neuronal vulnerabilities that underlie these complex disorders and draw parallels to the clinical phenotype, keeping in mind that these are systemic diseases. Throughout the chapter, we will comment on therapeutic feasibility for various targets introduced in the text and the ongoing efforts dedicated toward clinical translation. The reader is also referred to dedicated chapters in this compendium on HD (Chapter 17), FXS (Chapter 7), muscular dystrophy (Chapter 10), and ALS (Chapter 15) for more details.

Disease overview

Spinocerebellar ataxias (SCAs)

The spinocerebellar ataxias (SCAs) comprise a large group of disorders (48 at the time of this writing) that cause progressive ataxia, with an autosomal dominant (AD) inheritance. The naming of these disorders is somewhat misleading. Originally intended to describe pathological involvement of the cerebellum and spinal cord in these patients, involvement of both systems is not necessarily present nor sufficient to describe pathological processes inherent to each disease.[1] SCAs are organized in a numerical system, indicating the chronological order in which the locus or gene was described (SCA 1, 2, 3, and so forth).

The SCAs have an aggregated prevalence of 2.7 cases/100,000 individuals. Here, we focus on SCAs caused by REDs. As noted in Table 18.1, there are currently 12 repeat-expansion SCAs (SCAs 1, 2, 3, 6, 7, 8, 10, 12, 17, 31, 36, and 37). Six of these disorders (SCAs 1, 2, 3, 6, 7, 17) are caused by repeats of an exonic CAG codon encoding translated stretches of glutamine in the mutated protein, so-called polyglutamine expansion (polyQ) SCAs. All polyQ SCAs share accumulation and aggregation of the encoded protein in intraneuronal deposits, often within the nuclei of neurons.[2] Notably, although a CAG expansion occurs in SCA12, it is not included in this polyQ group since the mutation is a noncoding region. In SCA8, the CTG repeat is transcribed in both directions, and transcription of the antisense strand through repeat-associated non-ATG (RAN) translation can result in a polyglutamine-containing protein.[1]

The age of onset of most SCAs is in the third or fourth decade of life, but there is great variability. SCAs share a common feature of many REDs in which the size of the expansion correlates inversely with the age of onset. The most common initial motor symptom is ataxia (comprising imbalance with unsteadiness in gait and incoordination in fine motor tasks, speech, and oculomotor control). But many SCAs have noncerebellar neurological symptoms, including dementia, other movement disorders (parkinsonism, dystonia, chorea), pyramidal symptoms (weakness, spasticity), sleep disorders, peripheral neuropathy, and motor neuron disease. Some SCAs have specific, suggestive combinations of symptoms. For example, slow saccadic eye movements are seen in SCA2, parkinsonism is encountered in SCA3, visual loss with SCA7, seizures with SCA10, and neuropsychiatric changes and chorea with SCA17. **Cases 1–3 in** Boxes 18.1 and 18.2 illustrate different SCAs. Altogether, these polymorphic presentations suggest that the ataxin proteins encoded by SCA genes are critically important in nervous system function.

Other REDs that encode polyQ stretches include HD, SBMA, and DRPLA. HD and SBMA are extensively covered in other chapters, but we will briefly discuss DRPLA here.

Dentatorubro-pallidoluysian atrophy (DRPLA)

DRPLA is a condition named for the neuroanatomical distribution of the pathology, namely the dentatorubral (dentate nucleus in the cerebellum to the red nucleus in the midbrain) and pallidoluysian (globus pallidus and subthalamic nucleus) systems. It is an AD, inherited ataxia-plus syndrome caused by an abnormal CAG repeat in the *ATN1* gene, encoding for the atrophin-1 protein (ATN1). Most commonly, individuals present in the fourth or fifth decade with ataxia, chorea, and cognitive decline.[3] As with other polyQ diseases, the age of onset inversely correlates with repeat size. Juvenile onset, associated with larger repeats, may present with seizures, myoclonus, and neuropsychiatric symptoms, frequently later developing ataxia and cognitive decline. Although the disease is known to be most common in patients with Japanese ancestry (0.2–0.7/100,000 cases), it has also been observed in non-Asian populations. The disease imparts progressive disability. Individuals frequently become wheelchair-bound dependent on most daily activities and suffer premature death.

Friedreich's ataxia (FA or FRDA)

Friedreich's ataxia (also called FA or FRDA), named after the neurologist who first described the condition in the 19th century, is the most common autosomal recessive ataxia. It is most commonly caused by biallelic (affecting both alleles) trinucleotide (GAA) repeats in the first intron of the *FXN* gene, located on chromosome 9. The disease has a prevalence estimated at three cases/100,000 individuals.[4] Disease onset usually occurs in the second decade of life, with longer GAA repeats associated with

TABLE 18.1 Summary of repeat expansion disorders.

Disease	Gene, protein	Repeat, location	Normal repeat range	Pathologic repeat range	Clinical syndrome
SCA1	ATXN1, Ataxin-1	CAG, exon	25–36	41–81	Cerebellar ataxia; dysarthria ophthalmoparesis; dysphagia, amyotrophy; pyramidal signs; extrapyramidal signs
SCA2	ATXN2, Ataxin-2	CAG, exon	15–24	35–59	Cerebellar ataxia, ophthalmoplegia, peripheral neuropathy; motor neuron disease (intermediate length)
SCA3	ATXN3, Ataxin-3	CAG, exon	13–36	62–82	Cerebellar ataxia; ophthalmoparesis; parkinsonism; peripheral neuropathy
SCA6	CACNA1A, Cav2.1	CAG, exon	6–17	21–30	Pure cerebellar ataxia
SCA7	ATXN7, Ataxin-7	CAG, exon	7–27	37–460	Cerebellar ataxia; macular degeneration; ophthalmoplegia
SCA8	ATXN8, ATXN8OS[b]	CTG, ORF (ATXN8), 3′UTR (ATXN8OS)	15–50	80–250	Gait ataxia, dysarthria, dystonia, bradykinesia
SCA10	ATXN10, Ataxin-10	ATTCT, intron	10–32	800–4500	Cerebellar ataxia, dysarthria, dysphagia, epilepsy
SCA12	PPP2R2B, PP2A	CAG, 5′UTR	7–32	51–78	Cerebellar ataxia, hyperreflexia, parkinsonism
SCA17	IFRD1, TATA-box binding protein	CAG, exon	25–40	49–66	Cerebellar ataxia, dementia, dystonia, chorea, psychiatric symptoms
SCA31	BEAN1, BEAN1	TGGAA, intron	0 (mutation is an insertion of repeat)	[a]	Cerebellar ataxia, dysarthria, nystagmus, sensorineural hearing loss
SCA36	NOP56, nucleolar protein 56	GGCCTG, intron	3–14	>650	Cerebellar ataxia, hyperreflexia, dysarthria, atrophy, fasciculations
SCA37	*DAB1*	ATTTC, intron	0 (mutation is an insertion of repeat)	31–75	Cerebellar ataxia, dysarthria, oscillopsia
DRPLA	ATN1, Atrophin-1	CAG, exon	6–35	48–93	Cerebellar ataxia, myoclonus, seizures, cognitive decline
Huntington's disease	HTT, huntington	CAG, exon	<27	>36	Chorea, athetosis, dementia, psychiatric symptoms
Friedreich's ataxia	FXN, frataxin	GAA, intron	<30	100–1300 (majority >400)	Gait ataxia, dysarthria, dysphagia, scoliosis, cardiomyopathy
Fragile X syndrome, FXTAS	FMR1, FMRP	CGG, 5′UTR	5–44	55–200 (FXTAS), >200 (FXS)	FXTAS: Tremor, ataxia; FXS: Dementia, developmental delay

[a] *Repeats too complex to sequence reliably.*
[b] *ATXN8 and ATXN8OS are overlapping genes on sense and antisense strands.*

BOX 18.1 Clinical case scenarios

Case 1:
A 48-year-old woman presented to the clinic with trouble walking. About 4–5 years ago, she started having balance difficulties and slurred speech. More recently, she noticed a hand tremor in her left hand. Her father had balance problems with onset in his 60s, and her paternal uncle had amyotrophic lateral sclerosis (ALS). On examination, she has slow saccadic eye movements and dysmetria in both upper extremities and walks with a widened, ataxic gait. Her reflexes are brisk throughout, and there is mild bradykinesia and rigidity in her left hand.

Case 2:
A 53-year-old man presented for evaluation of progressive gait imbalance over the past 10 years. He struggles on uneven surfaces and feels off balance while in the shower. He also has an involuntary tremor in his hands, worse on the right. His sister had onset of balance difficulties in her 50s, and her daughter appears affected too. On examination, he demonstrates dysarthric speech, nystagmus, asymmetric bradykinesia with an intermittent rest tremor, peripheral neuropathy, appendicular dysmetria, and an ataxic gait.

Case 3:
A 52-year-old woman presented to the clinic for evaluation of gait imbalance. Over the past 15 years, she has noticed gradual difficulty with ambulation, blurry vision, weakness, and numbness and tinging in her lower extremities. Recently, she developed urinary incontinence. Her family is from South Africa. Her mother had an unstable gait with frequent falls; her brother became blind at age 38; and her son has seizures. On examination, she has moderately reduced visual acuity, dysarthric speech, weakness in an upper motor neuron pattern, brisk reflexes, peripheral neuropathy, and prominent appendicular ataxia.

Case 4:
A 20-year-old man complains of progressive staggering gait and speech. Over the course of just a few years, he declined from a healthy track athlete to requiring a cane. He recently developed shortness of breath on exertion. He has no neurological family history. His examination demonstrates a systolic murmur at the apex, nystagmus, dysarthria, areflexia, sensory loss to vibration and proprioception in his lower extremities, and an ataxic gait. He also has pes cavus and scoliosis.

BOX 18.2 Return to clinical case scenarios

Case 1:
The patient's initial concern of gait difficulty is a common reason to present to a neurological disorders clinic, but it is rather nonspecific. Her examination of ataxia with some parkinsonism engenders a broad differential and could even be a presentation of multiple system atrophy (MSA). But her family history of an uncle with ALS is somewhat peculiar. A repeat expansion panel returned positive for 39 repeats in *ATXN2,* diagnostic of SCA2.

Case 2:
Similar to Case 1, this patient presented with gait instability and shared many clinical features including ataxia and parkinsonism. Ultimately, this patient's combined clinical course and family history prompted testing for an inherited ataxia, with a repeat panel ultimately returning positive for 68 repeats in *ATXN3,* diagnostic of SCA3 (MJD).

Case 3:
The patient in Case 3 harbors similarities to Cases 1 and 2, but has added features of motor weakness and, most uniquely, acquired visual impairment. Patient 3 was diagnosed with SCA7 based on 72 repeats in *ATXN7.*

Case 4:
Due to the leg swelling and dyspnea in Case 4, the patient underwent cardiac evaluation and was diagnosed with dilated cardiomyopathy. Despite the lack of family history, the patient's progressive course prompted testing for genetic ataxias, and he was found to be homozygous for GAA repeats (732 and 999) in the frataxin gene (diagnostic of FRDA).

younger onset. However, onset as late as the fifth or sixth decade may occur, especially with shorter repeats.[5,6] Atypical presentations result from compound heterozygous mutations where one allele contains an expansion and the other allele has single nucleotide or copy-number variants.

Clinically, patients often present with clumsiness, scoliosis, and peripheral neuropathy causing decreased reflexes, loss of proprioceptive and vibratory sensation, and a particular clawed deformity of the foot named "pes cavus." Oculomotor abnormalities associated with cerebellar dysfunction include ocular dysmetria (inaccuracy), abnormal saccades, nystagmus, and square-wave jerks (involuntary horizontal refixation ocular movements). FRDA is a systemic disease, and some patients may develop cardiomyopathy concomitant with or even preceding the ataxia. Despite the peripheral neuropathy, many patients have an extensor plantar reflex (Babinski sign), indicating involvement of

the pyramidal tracts. In advanced stages of the disease, corticospinal tract involvement becomes more evident by progressive weakness and spasticity. As disease advances, diabetes mellitus, optic atrophy, and auditory neuropathy may occur.[7] As noted above, atypical phenotypes may occur with delayed onset, named late-onset (LOFA, defined as onset >25 years old) and very late-onset (VLOFA, defined as onset >40). These patients also exhibit gait and limb ataxia (lack of coordination), but the disease progresses slowly and pyramidal signs may be present early. In initial stages, reflexes may be preserved. Nonneurological features, including cardiomyopathy, scoliosis, pes cavus, and diabetes, are infrequent in atypical phenotypes. **Case 4** is an example of FRDA.

Fragile X syndrome, fragile X tremor ataxia syndrome, and fragile X premature ovarian insufficiency (FXS, FXTAS, and FXPOI)

Fragile X is named after the folate-sensitive fragile site at the FRAXA locus on the X chromosome.[8] This group of diseases offers insight on the pleiotropic nature of repeat expansions in the *FMR1* gene. Normal subjects have between 5 and 40 repeats of a CGG triplet in the promoter region of the *FMR1* gene. Individuals with >200 repeats develop Fragile X Syndrome, a common cause of developmental delay and intellectual disability occurring in 1/5000 males.[9] Additional phenotypic features include neuropsychiatric symptoms autism (Chapter 5), anxiety, ADHD (Chapter 3), prominent ears and an elongated face, and macroorchidism (enlarged testis) during puberty. Repeat lengths in the intermediate range (55−200 triplets) lead to variant phenotypes including Fragile X Tremor Ataxia syndrome (FXTAS) and Fragile X premature ovarian failure (FXPOI). FXTAS occurs mostly in males with mean onset in the seventh decade.[10] The disease prevalence is not well studied. Initial symptoms include action and intention tremor followed by gait ataxia. Some patients may have associated parkinsonism, neuropathy, and executive dysfunction. Females may have milder symptoms because of X-chromosome inactivation. Diagnostically, brain MRI classically demonstrates white matter lesions in the middle cerebellar peduncle, splenium of the corpus callosum, or subcortical regions. In women, FXPOI causes reduced fertility, menstrual irregularities, and premature cessation of menses (i.e., under age 40) (see Chapter 7).

Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS)

CANVAS is an adult-onset, slowly progressive disorder characterized by imbalance, sensory neuropathy (neuronopathy), bilateral vestibulopathy, chronic cough, and occasional autonomic dysfunction.[11,12] Recently, investigations in familial cases of CANVAS syndrome uncovered a recessive intronic AAGGG repeat expansion in the replication factor C subunit 1 (*RFC1*) gene, differing from the normally occurring AAAAG allele.[13] The disease has a mean age of onset in the sixth decade (ranges from 30 to 80 years old) and often begins with gait ataxia and peripheral neuropathy, with later appearance of visual disturbances, dysarthria, and dysphagia. Curiously, many patients have a dry spasmodic cough that can be an early finding. Early reports have suggested that repeat expansion in *RFC1* is present in all familial CANVAS cases and in at least 70%−90% of sporadic cases.[13,14]

Cellular and animal models of disease

There are various cellular and animal models used in the field to study REDs, and each of these models has benefits and limitations as we attempt to replicate human disease in the laboratory.

The simplest models for polyglutamine expansion disorders are tractable model organisms. The largest number of studies have been performed in yeast (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*), worms (*C. elegans*), and fruit flies. These model systems have been extensively reviewed elsewhere.[15−19] These models offer amenability for high-throughput genetic and small-molecule screens and have been invaluable in characterizing the genetic modifier space of polyQ-associated druggable targets, a number of which are now advancing to clinical trials.[20] Where genes are evolutionarily conserved, these organisms offer unprecedented opportunities to identify gene function. For instance, the function of the frataxin protein implicated in Friedreich's ataxia was first identified through investigation of the Yfh1 protein in yeast.[21]

Nevertheless, these models have several limitations. Yeasts provide limited insight into intercellular mechanisms of neurodegeneration, as they do not have a structured nervous system. Worms and fruit flies can be used to model glioneuronal interactions, but many genes implicated in human disease are not conserved in these organisms. Vertebrate models, such as zebrafish and mouse, thus remain highly relevant.[22−24] Mouse models offer mammalian homology with robust behavioral studies, but are lower throughput. Nonhuman primates[25] offer the closest intact whole-animal model organism in which to study REDs. In general, animal models provide a multisystem platform to study and manipulate effects of CNS diseases, with complex behavioral and cellular pathology outcomes.

Within these disease models, replicating the genetics of repeat expansion has proven nontrivial due to the unstable nature of long nucleotide repeats and the difficulty of standard approaches for genome editing. Broadly speaking, three genetic approaches are used: (1) knockout (zero functional copies of the gene in question), (2) transgenic overexpression (randomly inserting a transgene encoding an expanded allele into the genome in addition to two wild-type copies of the gene), and (3) knock-in (creating a repeat expansion at the endogenous locus, in either a single or both alleles). Knockout models[26,27] are crucial for determining the necessity of a gene and its function in a complex system but often do not replicate the human disease where a toxic gain of function is thought to be the primary pathology driver. Transgenic models add in the repeat expansion and overexpression can be constitutive—always expressed under a specific promoter through development—or conditional—expression

is triggered through an exogenous ligand at a specified time. The latter has been helpful in determining the reversibility of phenotypes with therapeutic intervention. Transgenic models often portray similar cellular pathology, but nonphysiologic expression levels raise some doubts about human relevance. Knock-in mammalian models therefore best replicate human disease, but often require significantly longer repeats than reported in humans to replicate the phenotype seen in patients.[28,29]

To address some of the shortcomings of animal models and directly study human neurodegenerative disease, great strides have been made using human induced pluripotent stem cells (iPSCs)[30] from RED patients. An emerging literature suggests that critical human biology is not captured in the mouse,[31] and indeed reversal of neurodegenerative phenotypes in mouse models has not thus far been predictive of clinical success, underscoring the need for human models. The promise of iPSCs and differentiating them into various neuronal and nonneuronal subtypes is to model neurodegeneration in a dish. However, neurodegenerative diseases do not affect a single cellular population (as we comment on below), and thus, a significant challenge in the iPSC field remains recombining cellular subtypes into reproducible and robust mixed cellular cultures and 3D-organoids. Moreover, iPSC-derived cultures tend to approximate fetal maturity, and all epigenetic marks of aging (or environment) are removed during reprogramming from fibroblasts to iPSC. It will become important to develop methodology to accelerate aging in cultured cells or to find alternative methods of reprogramming. For example, direct transdifferentiation of fibroblasts to neurons may preserve age-related phenotypes better, as has been demonstrated in cellular models of HD.[32]

No single model offers a perfect replication of human REDs, but each offers unique insights into the biological underpinnings. As discussed below, combination of these models is requisite, and those themes conserved across multiple model systems are most likely to be appropriate targets for therapeutic intervention.

Pathophysiological mechanisms

DNA level

Repeat length

Trinucleotide repeat expansions are the most common and extensively studied REDs, particularly those with CAG repeat encoding for a polyglutamine stretch. This group includes HD (the most common inherited Mendelian neurodegenerative disease) and several ataxic disorders (SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, FRDA, and DRPLA). Longer polynucleotide repeats are also associated with disease, including tetranucleotide (Myotonic Dystrophy type 2 or DM2), pentanucleotide (SCA10, SCA31, SCA37, and CANVAS), hexanucleotide (C9ORF72 or SCA36), and even dodecamer repeats (Unverricht–Lundborg disease or EPM1). Many (but not all) trinucleotide repeats occur in protein-coding regions, while longer polynucleotides are found in noncoding regions, introns, or flanking untranslated sequences (Fig. 18.1).

Repeat locus

The precise location of the repeat expansion within a gene exerts a significant effect on the biology of REDs. Repeats in the 5′UTR or near the promoter region may alter gene expression (SCA12, FXS/FXTAS, EPM1).[9,33–35] Intronic repeats can disrupt splicing (C9ORF72, SCA8), leading to aberrant exclusion or inclusion of exons.[36,37] Repeats located in the 3′UTR (e.g., Myotonic Dystrophy type 1 or DM1) can disrupt transcriptional regulation by sequestering RNA-binding proteins.[38] Repeats located in exons are translated (for example, polyglutamines in HD and some SCAs, DRPLA; polyalanine in OPMD; polyleucine in HD-like-2), potentially altering function of the native protein and exerting deleterious effects through means described below. In addition, as further discussed, epigenetic changes can superimpose at these loci (FRDA, FXS/FXTAS).

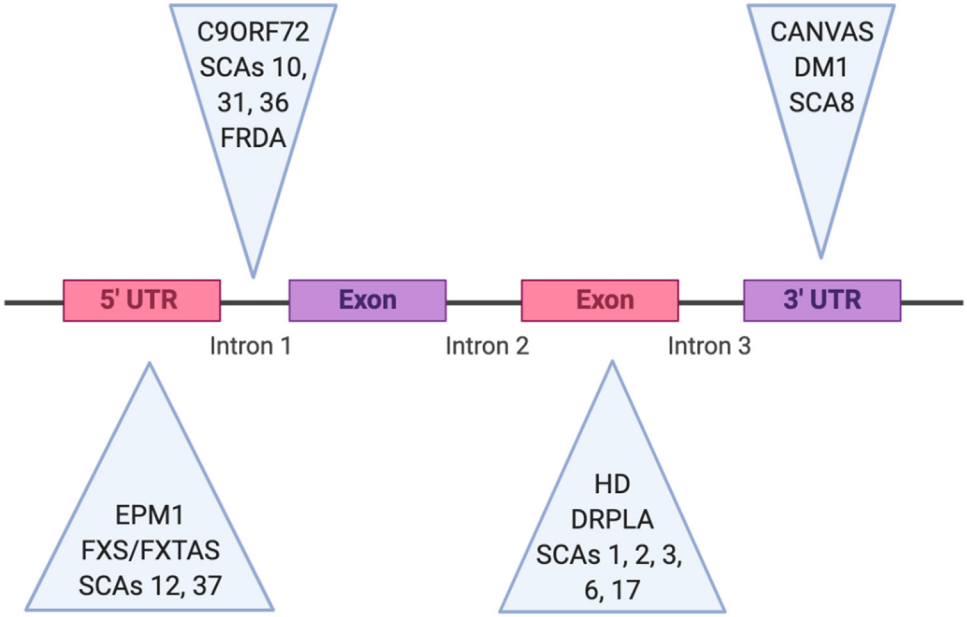

FIGURE 18.1 Genetic loci for various REDs. While the most common REDs are in coding exons and generate polyQ stretches, several others occur in noncoding regions. Expansions there can affect expression via disruption of splicing, sequestering RNA binding proteins, epigenetic modifications, or lead to aberrant proteins via repeat associated non-ATG (RAN) translation.

Expansion size

For most REDs, repeat expansion size drives one of three states: normal physiology, a preclinical (also called "premutation" or "carrier") state, and full-fledged disease manifestation. Longer repeats drive earlier symptom onset and can be associated with expedited progression. Additionally, longer repeats frequently lead to earlier symptom onset and can be associated with expedited progression. This is best documented in HD, SCAs 1, 2, 3, 6, 7 and DRPLA.[39–43] These diseases are also associated with *anticipation*, whereby each subsequent generation in a family displays longer repeats than the parental line. This phenomenon is presumed to occur in part because tandem repeats cause incorrect pairing of complementary DNA strands during replication. Thus, in familial cases, the subsequent generation may have earlier disease onset with more severe manifestations. Occasionally, de novo cases may occur when the expansion size crosses the threshold from the carrier/premutation state in the previous generation to full-fledged disease manifestation in the offspring. Beyond intergenerational differences, there is also intraindividual variation in the same generation within a particular pedigree. This results from somatic instability, a phenomenon in which altered repeat lengths are found within various tissues.[44–46] There is also evidence in HD and FRDA to suggest that repeat expansion number may increase with aging.[47–49]. (For the inheritance mode of anticipation, see below).

Despite having some clinical utility, in reality the distinction between normal, premutation, and disease-manifesting carriers is blurred. For example:

1. *Premutation state can be associated with pathologic phenotypes*: in HD, older patients with premutation length repeats demonstrate increased chorea scores and cognitive decline compared to age-matched controls.[50]
2. *Intermediate-length expansions can lead to a separate disease state through altered pathophysiology.* This is exemplified with expansions in the *FMR1* gene, where the repeat is located close to the promoter region. Large expansions (>200 repeats) lead to hypermethylation in an adjacent CpG island, leading to loss of *FMR1* expression. Affected individuals have FXS, manifesting in males with intellectual disability, autistic spectrum disorder, macroorchidism, and distinctive facial features. Affected females can have symptoms but with reduced penetrance due to random X inactivation. Repeats in the intermediate range (50–200 repeats) do not cause abnormal promoter methylation. In fact, individuals with intermediate expansions actually demonstrate increased *FMR1* mRNA expression (but decreased FMR protein levels due to inefficient translation). The increased mRNA levels drive pathology through a toxic RNA gain of function, sequestering RNA-splicing factors and driving the aberrant production of aggregation-prone proteins.[8] Effectively, there is inefficient translation resulting in decreased FMR protein levels. The phenotype of premutation carriers includes Fragile X Tremor Ataxia Syndrome (FXTAS, a condition occurring mostly in males in late-life with intention tremor, cerebellar ataxia, parkinsonism, and cognitive decline) and Fragile X-associated primary ovarian insufficiency (FXPOI, comprising menstrual cycle irregularity, decreased fertility, and earlier menopause).[51] Given the X-linked dominant inheritance of FMR1 expansions and anticipation, FXTAS may present in the maternal grandfather of a boy affected with FXS, while the mother and/or other female relatives in the pedigree may harbor ovarian failure (for more details, see Chapter 7).

   Expansions in ATXN2 provide an additional example. When individuals have >37–39 repeats, they develop symptoms of SCA2. At 33–34 repeats, there is reduced penetrance and/or late onset of disease. Intermediate lengths (29–33 repeats) do not lead to SCA2, but rather confer elevated risk for amyotrophic lateral sclerosis (ALS).[52,53] *ATXN2* encodes a key RNA-binding protein. Isoforms with intermediate repeat expansions modify the toxicity of other proteins mutated in familial ALS (TDP-43 and FUS).[54,55] The ATXN2-TDP-43 interaction appears to proceed through an RNA bridge; in both TDP-43 and FUS, intermediate polyQ ATXN2 leads to mislocalization of the proteins from the nucleus to the cytosol or ER/Golgi, respectively. Inappropriate protein–protein or protein–RNA interaction is implicated in downstream dysfunction in RNA processing and stress granule formation, perturbed Golgi function, and early apoptotic cell death. Intriguingly, in SCA2 multiple neuronal populations degenerate, including lower motor neurons.
3. *Disease-manifesting repeat sizes may lead to different disease manifestations*: There are rare reports of pathologically confirmed multiple system atrophy (MSA) in patients with pathologic expansions in ataxin genes.[56] The connection in these cases currently remains anecdotal because population-based genetic analyses have failed to convincingly show causal association of these pathologic expansions with these diseases.

Expansion sequence characteristics

Interruptions of an expanded repeat (e.g., CAT or CAA in the middle of a trinucleotide CAG repeat) may also reduce disease penetrance and slow disease progression, occasionally changing the disease phenotype and conferring additional risks.[57] For example, repeat interruptions in *ATXN2* (SCA2), *ATXN10* (SCA10), and *ATXN17* (SCA17) can lead to parkinsonian phenotypes.[58–62] The association of intermediate polyQ repeats in *ATXN2* with ALS mentioned above is also dependent on CAA interruptions.[63] Similarly, the number of CAA interruptions in *ATXN10* dramatically increases the risk of developing epilepsy.[64]

Mechanistically, CAA or CAU interruptions may change the hairpin RNA structure.[65] These altered hairpin structures have variable association with RNA-binding proteins and can therefore lead to disruption of posttranscriptional regulation of other genes.

Epigenetic modifications

Repeat expansions also modulate and are influenced by epigenetic modifications. As described above, *FMR1* expansions above a certain size lead to hypermethylation of critical CpG islands in the gene's promoter region, resulting in heterochromatin formation. Hypermethylation also occurs in pathologic repeat expansion carriers of the *FXN* gene (FRDA). The GAA expansion in *FXN* induces heterochromatin (compact chromatin structure) formation, via histone H3K9, H3K27, and H3K36

methylation. There is decreased acetylation of histone lysine residues near expansions in *FXN, HTT,* and *ATN1*.[66–68] The CTG expansion in *ATXN8OS,* a gene that overlaps with ATXN8, is associated with H3K9 dimethylation and reduced H3 acetylation.[69] The consequences of these epigenetic modifications remain unclear, but likely include transcriptional dysfunction and altered expression. Epigenetic machinery represents a tractable target for disease modification. Histone deacetylase (HDAC) inhibitors have been trialed in FRDA and HD and have been considered in DRPLA.[70]

Inheritance patterns and dichotomous gain/loss of function mechanisms

Most repeat expansion disorders are AD. The remainder are autosomal recessive (AR) or X-linked. Conventional wisdom supports a gain-of-function mechanism in AD REDs. The dominant inheritance pattern and phenocopy of key disease features in transgenic overexpression animal models support this idea. But loss of function is also implicated in AD REDs, in two specific ways. First, dominant negative mechanisms have been observed in an HD model where the mutant allele leads to dysfunction of the wild-type allele.[71] (For other potential loss of function in HD, see Chapter 17). Second, mislocalization of a pathologically expanded protein and sequestration of its native interaction partners can result in a loss of function at the same time as aberrant interactions can lead to a "toxic gain of function." Polyglutamine expansion of ATXN1, for example, is associated with reduced interaction with a native interacting protein, Capicua. This leads to a partial loss-of-function effect.[72]

In AR REDs, the proposed mechanism is loss of function. This is exemplified by FRDA, where about 96% of cases result from a 90−1300 GAA trinucleotide expansion within intron 1 of both *FXN* alleles. This leads to subsequent heterochromatin formation as noted above. The remaining 4% of patients have a point mutation on one allele combined with an expansion on the other. FXN expression levels in these patients are greater than in those with biallelic repeat expansions, but still insufficient for normal protein function. While there is some variability with age of onset of ataxia and presence of associated disease manifestations (e.g., diabetes mellitus, cardiomyopathy), the ataxic phenotype is quite similar between the two genetic etiologies.[73] A similar mechanism underlies EPM1, a neurodegenerative condition presenting with stimulus-induced myoclonus and tonic−clonic seizures, later developing ataxia, dysarthria, and an intention tremor. EPM1 can also develop from either a biallelic CCC−CGC−CCC−GCG dodecamer repeat expansion (>30 repeats) in the cystatin B (*CSTB*) gene or a compound heterozygote of a point mutation combined with an expanded allele.[74–76]

Lastly, there is a relationship between sex of the affected parent and the expansion stability. Exonic repeats have a higher propensity for intergenerational expansion (anticipation) if acquired from the father while noncoding REDs show greater anticipation when maternally inherited.[77] The etiology for this pattern is unclear but may be related to meiosis during spermatogenesis.

Subcellular level: RNA, proteins, organelles

Multiple areas of subcellular dysfunction are implicated in REDs (Fig. 18.2). Below, we discuss several of the best characterized and comment on therapeutics that have been developed against these potential targets.

RNA-mediated mechanisms

Although a majority of preclinical studies for REDs have focused on alterations at the protein level (discussed below), aberrations in RNA regulation also contribute to disease pathology.[78–80] In an early fly study, CAA interruptions of the *ATXN3* CAG expansion dramatically reduced toxicity, despite the creation of an identical length polyQ protein.[79] In this study, modification of toxicity by the RNA-binding protein (RBP) mbl also implicated toxicity at the RNA level. Repeat-mediated RNA toxicity is partly mediated by sequestration of RBPs, resulting in alternative splicing or translation in other genes. This was originally described in DM1,[81,82] but has been widely implicated in other REDs.[37,80,83–87] In addition, repeat-expansion harboring RNAs may be directly toxic themselves through the formation of RNA foci.[38,80,88–90]

Protein aggregation

As in other neurodegenerative disorders, protein aggregates in REDs range from insoluble fibrils to smaller, soluble oligomers. There are two main theories of aggregate formation: (1) nucleation-elongation theory,[91,92] where polyQ monomeric peptides are in rapid equilibrium with a β-sheet that serves as a template for stacking additional β-sheets, and (2) association-conformation theory,[93] where hydrophobic interactions between monomers seed smaller, soluble oligomers that eventually propagate to larger fibrils. The exact kinetics of aggregation seem to vary based on polyQ length with longer repeats showing faster aggregation.[94,95]

While most protein aggregates occur because of an exonic repeat expansion, repeats in noncoding regions can also lead to aberrant translation and eventual aggregation. For example, in SCA8, the *ATXN8* gene overlaps with the *ATXN8OS* gene on the complementary antisense strand. Despite the repeat expansion in *ATXN8* occurring in a noncoding region, there is evidence of expression and translation of polyQ-expanded *ATXN8OS*. PolyQ aggregates are also seen in SCA8 brains.[96] Additionally, repeat expansions in RNA can form hairpin structures, from which non-ATG translation can initiate in multiple reading frames, resulting in accumulation of (typically) nonsensical protein. This is termed repeat-associated non-ATG (RAN) translation. First discovered in SCA8,[97] RAN translation has been uncovered in *C9ORF72*[98,99], SCA3,[100] and FXTAS.[101]

Once formed, polyQ aggregates can spread between cells in a prion-like fashion.[102] Although REDs stem from ubiquitous mutations, each cell has varying capacity for proteostasis.[103] Thus, prion-like spread of aggregates from more susceptible cells to less may explain the temporal and spatial evolution of REDs.

Whether these amyloid-like fibrils are toxic themselves or mere bystanders of toxicity remains a pressing question in REDs.

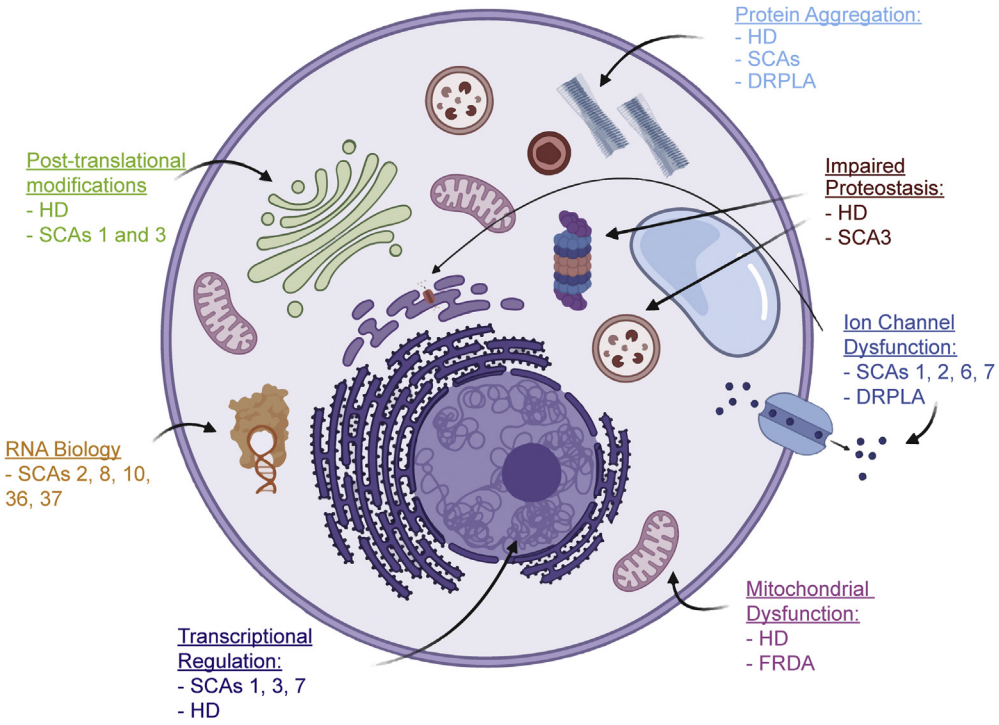

FIGURE 18.2 Subcellular dysfunction drives pathogenesis of REDs. Various subcellular organelles and pathways have been implicated among REDs. Here, for simplicity, we focus on the perturbations best described in the context of specific genes and most specifically tied to perturbations of their respective endogenous functions. Several of these pathways represent therapeutic targets for disease intervention.

For instance, the soluble oligomeric forms of polyQ and other RED aggregates may directly be cytotoxic,[104–107] but an early study of HTT protein aggregation showed that neurons with large aggregates were protected against toxicity.[108] It is therefore likely that a plethora of inclusions, with discrete biological consequences, form in these diseases.

Posttranslational processing

Posttranslational processing of polyQ proteins affects their aggregation. Among the best studied modifications are phosphorylation, ubiquitination, and posttranslational cleavage. For example, phosphorylation of ATXN1 at S776 and ubiquitination at K589 lead to reduced aggregation.[109,110] Cleavage of ATXN3 and HTT by caspases and calpains is crucial for disease phenotype.[111–114] Several other polyQ proteins (ATXN7, ATN1, and the androgen receptor) are caspase substrates and cleavage promotes cytotoxicity of the aggregated products.[115–117] Mutation of the caspase cleavage site in ATN1 (Asp109) results in reduced aggregation and lowered cytotoxicity. In SCA3 cell and mouse models, treatment with a caspase inhibitor suppressed ATXN3 aggregation. While these data prompt consideration of such compounds for therapeutic intervention in REDs, selectively targeting their pathologic rather than physiologic function remains a formidable challenge.

Protein homeostasis

Major cellular protein-degradation pathways include the ubiquitin-proteasome pathway (UPS) and autophagy. Both pathways are impacted in REDs. The ubiquitin-binding protein p62/SQSTM1 delivers ubiquitinated cargoes for both autophagy and UPS. p62 coaggregates with HTT[118] and ATXN3[119] in disease models and has been used as a surrogate of ineffective clearance of these misfolded proteins Moreover, aggregation of *HTT* increases with inhibition of autophagy and decreases with autophagic inducers, such as the mTOR inhibitor, rapamycin.[120] The same is true for ATXN3 and ATN1.[121,122] Interestingly, while behavioral phenotypes in an Atxn3 model were rescued, the same was not true for Atn1.[123] Protein clearance is an intervention being pursued in several studies[124,125] (Clinical trial NCT03932669 is examining effect of an autophagic inducer, Nilotinib, on ataxia), though the autophagy-induced dissociation of aggregation and behavioral phenotypes seen in some contexts suggests that aggregation may not be a straightforward therapeutic target.

Subcellular localization

In conjunction with protein aggregation, subcellular localization plays a key role in driving disease phenotype. For example, in SCA1 and SCA3, the polyQ protein must be localized to the nucleus to drive disease.[126,127] In these models, when the nuclear localization signal sequence is mutated or the protein tagged with a nuclear export signal, cellular pathology is not seen regardless of polyQ length. Conversely, CAG expansion in the *ATN1* gene contributes to the increased proteolytic processing of the ATN1 protein and translocation of the CAG expansion-containing C-terminal fragment to the cytoplasm, seeding aggregation.[128]

Downstream effects

DNA damage response

Cellular models of HD show accumulation of single- and double-strand DNA breaks.[129] Overexpression of DNA damage repair components ameliorates the HD pathology and behavior in mouse models.[130] Lastly, a large genome-wide association study (GWAS) showed that DNA repair pathways are critical modifiers of age of onset in HD.[131] Similar themes have emerged in REDs. For example, polyQ ATXN1 interacts with the DNA repair protein high-mobility group Box 18.1 (HMGB1) and impairs its native function.[132] Viral delivery of HMGB1 ameliorates the SCA1 phenotype in an *ATXN1*-knockin mouse.[133] Mismatch repair machinery also protects against intergenerational instability of the GAA repeat in FRDA.[134]

Mitochondrial dysfunction

Dysfunctional mitochondria have been observed in REDs, best described in FRDA and HD. In FRDA, the normal frataxin protein associates with the inner mitochondrial membrane, putatively affecting iron-sulfur cluster biogenesis. This function is highly conserved across evolution to yeast.[15,135] Reduced levels of frataxin have been tied to increased oxidative stress.[136] In HD, there is decreased activity of mitochondrial complexes II and III. The resulting shift toward anaerobic metabolism leads to increased lactate, which can be observed by magnetic resonance spectroscopy in early HD patient brains.[137]

Ion channel dysfunction

Several polyQ SCAs demonstrate abnormal electrophysiologic activity in Purkinje cells, the major motor output of the human cerebellum. Aberrant spiking activity has been demonstrated in models of SCAs 1, 2, 6, and 7.[138–141] This irregular firing correlates with behavioral phenotypes in those models and even precedes cerebellar degeneration. Of note, this is not the case in SCA3, where irregular spiking does not correlate with motor symptoms in later disease stages.[142]

The abnormal firing pattern largely stems from reduced expression of potassium ion channels or the calcium channels sourcing those calcium-activated potassium channels. In SCA1, potassium channel expression appears to be directly transcriptionally repressed, though this is not clear in other SCAs. Restoring potassium channel activity in those SCA models, either by genetic overexpression or by small-molecule activators, returns proper spiking, improves motor symptoms, and delays cellular degeneration.[138–141,143] Early promising compounds are transitioning toward the clinic,[144] with more thorough discovery efforts already underway.

Cellular level

Pathological changes in the central nervous system

Within the central nervous system, REDs clearly impact neurons, as demonstrated by intracellular proteinaceous aggregates accompanied by dendritic atrophy, axonal swelling, and synaptic loss. However, nonneuronal cells are affected as well. In individuals with C9ORF72 expansion, microglia show similar p62 inclusions as diseased neurons and contribute to neuronal toxicity in cellular coculture models.[145] Mutant HTT is expressed in immune cells at high levels, and microglia activation and reactive gliosis are prominent findings in HD.[146,147] Over- or inappropriate microglia activation can induce an inflammatory cascade that leads to caspase activation, free radical production, and subsequent neurodegeneration.[148] Microglia activation also seems to increase during disease progression and correlates with severity. Reactive astrocytosis and glial or microglial activation has been observed in several SCAs and DRPLA.[149–151]. (For more details on inflammation in neurodegenerative diseases, see Chapter 23).

When examining these cells as grouped populations, the specific cellular vulnerability of REDs tracks with clinical presentation (Fig. 18.3). Regions of common dysfunction or degeneration likely explain clinical overlap among REDs. SCA2 and SCA3 patients demonstrate parkinsonism, and histopathological evaluation shows degeneration of the substantia nigra pars compacta, the same population of dopamine neurons vulnerable in Parkinson's disease.[152,153] Cerebellar Purkinje cells are heavily affected in SCAs 1, 2, 6, 7, 8, and 17, contributing to clinical ataxia.[154,155] Interestingly, a similar phenotype can also be caused by distinct patterns of neuronal vulnerability. In SCA3, Purkinje cells are spared and instead brainstem-cerebellar circuits degenerate and underlie the ataxic phenotype.[142] Furthermore, uniquely affected brain regions lead to distinct clinical features of some SCAs. For example, striatal loss is a hallmark feature of early HD. Chorea, dementia, and neuropsychiatric symptoms are prominent features in HD and SCA17, whereas SCA7 uniquely displays retinal degeneration.

Pathological changes in the peripheral nervous system and elsewhere

Peripheral neuropathy

Sensory neuropathy is a major contributor to ataxia, and degeneration of the posterior columns of the spinal cord and dorsal root ganglion likely underlies these symptoms. This pattern is a hallmark of Friedreich's ataxia[156,157] but is also found in CANVAS[158] and several SCAs.[159–161] Pathophysiologically, ATXN3 has been shown to aggregate in the dorsal root ganglion and Schwann cells.[162] Clinically, peripheral neuropathy has been measured using nerve conduction studies, and results typically implicate a mixed axonal and demyelinating sensory neuropathy. There appears to be a relationship between the GAA repeat number in FRDA and nerve conduction studies (NCS) findings,[163] but this has not been replicated in other REDs. Longitudinal studies of peripheral nerve disease in REDs remain an untapped area for monitoring disease progression and have been limited, in part, due to the floor effect of NCS. More sensitive metrics, such as monofilament testing and in vivo reflective confocal microscopy, correlate well with motor symptoms in FRDA[164] and are an alternative for such studies in other REDs.

Myopathy

Significant histological changes on muscle biopsy have been described in DRPLA[165] with absence of type IIB (fast-twitch, high-oxidative) fibers and reduced diameter of all fibers. This is

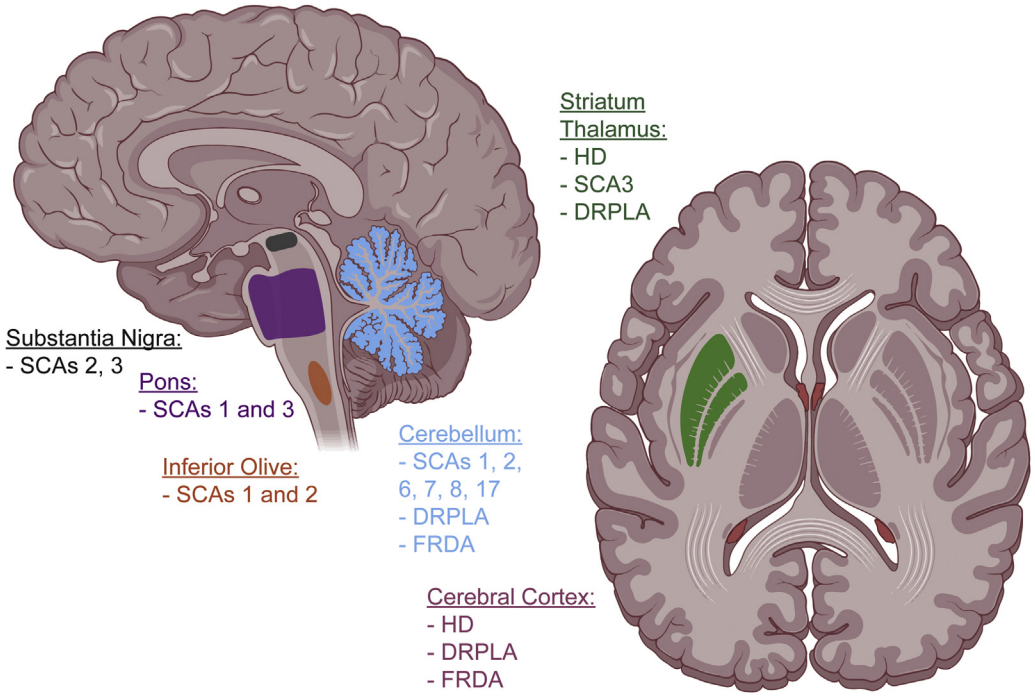

FIGURE 18.3 CNS distribution of REDs. Selective vulnerability, of neuronal and nonneuronal cell types, likely drives central manifestations of REDs.

associated with impaired mitochondrial function in muscle fibers.[166] Several SCA patients can also exhibit muscle wasting and atrophy later in the disease, but evidence for primary involvement is most clear in SCA17, where knock-in mouse models showed severe muscle atrophy.[167] Given neuronal vulnerability to polyglutamine aggregation, it was originally thought that muscle atrophy was due to motor neuron toxicity and lack of innervation. However, aggregates seen in muscle cells suggest a primary myopathic process.[168]

Gut microbiome

The effect of the gut microbiome has been linked to neuroinflammation in Parkinson's disease and Alzheimer's disease in both idiopathic and genetic forms of the disease.[169] Whether altered microbiome is causal to a disease process or consequence is still under investigation. But growing evidence suggests that, regardless of this, manipulating the microbiome may alter disease outcome in REDs. For example, composition of the microbiome modulates survival in *C9ORF72* mice.[170] Disruption of the gastrointestinal microbiome was observed in HD mouse models[171–173] and in HD expansion carriers and was associated with cognitive performance scores and clinical outcomes.[174] Ongoing studies focus on determining if dietary modifications can improve clinical outcome in a number of neurodegenerative diseases, with ketogenic, Mediterranean, and Omega3-rich diets showing some impact on cognitive function in animal studies.[175]

Cardiomyopathy

Nearly all patients with FRDA develop dilated cardiomyopathy during the course of the disease, as a consequence of mitochondrial damage.[176] Thus, FRDA patients should follow closely with a cardiologist and obtain annual echocardiograms. However, the effect of REDs on other cardiovascular pathologies, such as peripheral vasoconstriction or arrhythmias, has not been extensively explored.

Endocrinopathy

Patients with FRDA also suffer from an increased risk of impaired glucose tolerance leading to diabetes mellitus.[5] Mitochondrial sensing is crucial to pancreatic Beta cell stimulus-induced secretion of insulin. Thus, mitochondrial dysfunction driven by frataxin expansion is thought to underlie the development of insulin resistance in FRDA.[177,178]

In FXPOI, women develop primary ovarian insufficiency (POI). Interestingly, even within the intermediate repeat range in the *FMR1* gene (50–200 repeats), there is a nonlinear relationship with risk of POI.[179] Evidence suggests that POI in FXPOI is related to poor follicle growth and function rather than depletion.[180]

Psychiatric comorbidities

As with many neurodegenerative disorders, psychiatric disease is often comorbid with REDs. Psychiatric symptoms are a hallmark of HD, but depression and anxiety are also prominent features in FXTAS/FXPOI[181] and SCA17,[182,183] along with difficulties in attention and aggression. Early research into the genetics of primary psychiatric disease has revealed possible links to repeat expansions,[184] but these investigations are nascent.

Individual level

The clinical cases presented in this text illustrate the difficulty of making a definitive diagnosis, which is eventually confirmed via genetic testing (Box 18.2). There is substantial phenotypic

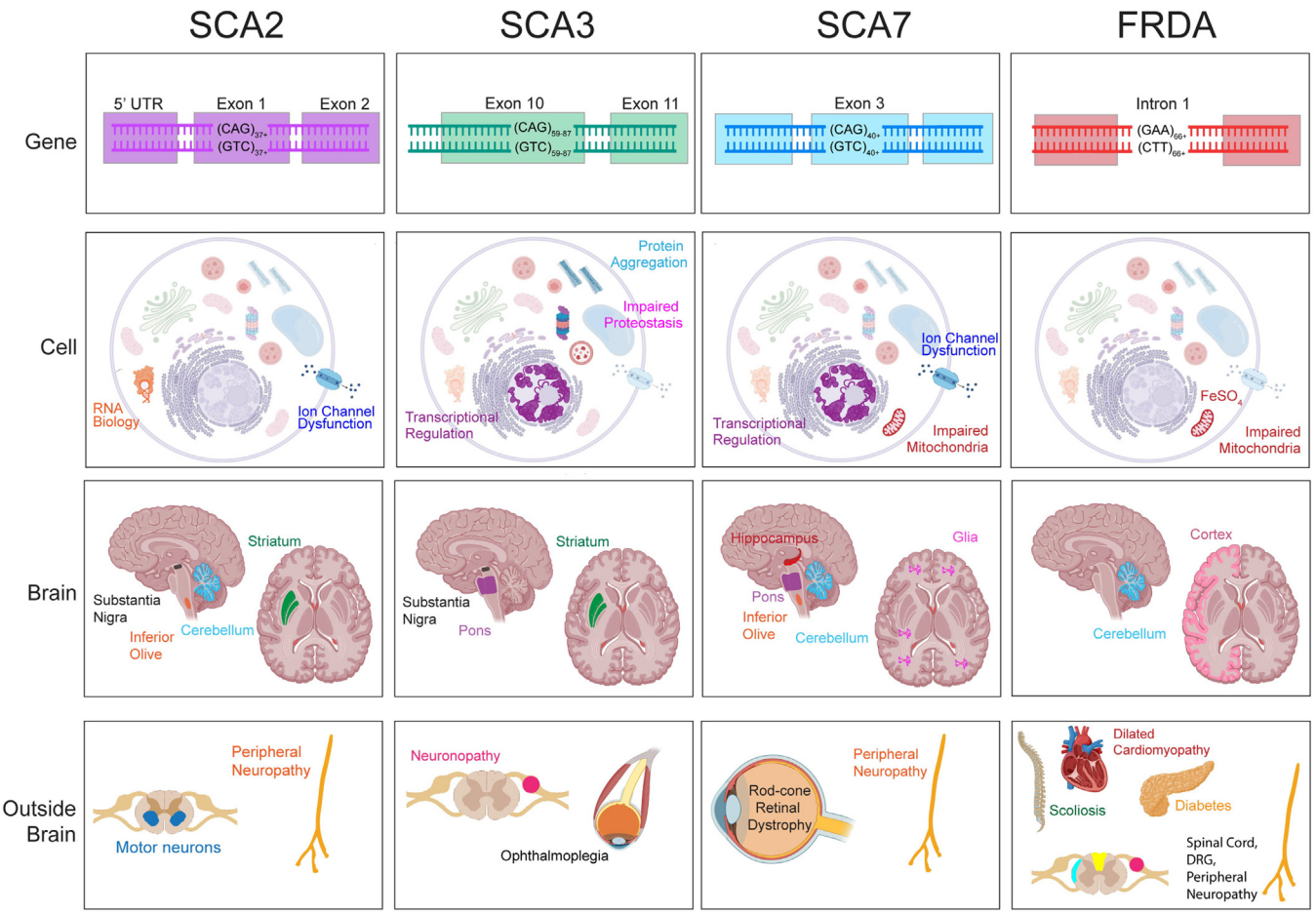

FIGURE 18.4 Comparison of multiple REDs across microscopic to macroscopic spectrum. Here represented are our clinical cases, from nucleotide level to systemic manifestations. Despite shared commonalities across all four diseases, there are unique drivers, which likely lead to distinctive clinical presentations. Finally, it is paramount to treat REDs as systemic disorders, with pathology both inside the brain and peripherally.

overlap that arises from the subcellular signatures and specific cellular vulnerabilities driven by each repeat expansion (Fig. 18.4). And while there are subregions of the CNS (i.e., cerebellum, brainstem, basal ganglia, spinal cord) that are particularly susceptible to toxicity from these expansions, there is a differing degree to which each disease results in neuronal degeneration, white matter loss, and glial involvement. For example, cerebellar Purkinje cells are more greatly affected in SCA's 1, 2, 6, 7, 8, and 17 as compared to SCA3. Occasionally, there are uniquely affected areas, such as the retinal degeneration observed in SCA7 that provides a diagnostic clue. However, unique phenotypes are the exception rather than the rule.

To address this phenotypic overlap when evaluating individuals suspected of having REDs, clinicians can resort to genetic panels that aggregate diseases with similar characteristics. However, physicians still need to be able to accurately identify the salient clinical features to guide and interpret testing. Specific laboratory techniques, distinct from next-generation sequencing (NGS), are required to identify expansion diseases, such as Southern blotting and/or repeat-primed PCR. Whole exome sequencing, an NGS-based technique, which analyses the coding regions of the human genome, has many blind spots: it does not pick up repeat expansions, mutations in intronic regions or changes in the DNA that preserve DNA sequence (e.g., copy number variations and/or aneuploidy, balanced translocations) and epigenetic modifications. Innovations in bioinformatic analysis and whole genome sequencing are emerging tools to clear these "blind spots," allowing for the potential identification of repeat expansions as well as mutations in noncoding regions and copy number variants.[185]

Therapeutics against repeat expansions

Not a single disease-modifying therapy exists for any repeat expansion disorder. This is remarkable and humbling in light of the fact that these are monogenic disorders, and a repeat expansion at the Huntingtin locus was discovered over 20 years ago. Each of the different molecular and cellular pathways outlined above is potentially druggable, and we direct the reader to the references cited in those sections. However, the monogenic nature of these disorders begs the question of their amenability to gene therapies. The notable success of antisense oligonucleotides (ASOs) and adeno-associated virus (AAV)-based approaches for spinal muscular atrophy (SMA)[186–188] and Duchenne Muscular Dystrophy (DMD),[189] and the exciting trend toward slowing down clinical decline in patients with a genetic form of ALS

through ASO-mediated SOD1 reduction (Phase I/II clinical trial NCT02623699), all raise the possibility that complex neurodegenerative and neuromuscular diseases are amenable to genetic therapies. We have thus decided to focus below on therapies directed against the implicated gene or expansion, either based on gene therapy or pharmacological approaches, in the remainder of this review.

The challenge of nucleotide-targeting therapies in REDs cannot be overstated. The genetic lesions are complex and difficult to alter. More fundamentally, it remains unclear whether these diseases are clearly loss or gain of function. Thus, high level overexpression or a full knockdown of a transcript may not be the ideal treatment strategy. Moreover, expansion repeats are scattered throughout the genome and guiding the specificity of these therapies will be challenging. Despite these challenges, as the underlying cause lies in a genetic expansion, tightly controlled nucleotide-targeting therapy remains an attractive strategy for REDs.

Genetic therapies

Innovations in genetics have allowed for therapeutic advances in treating REDs, namely with two major approaches: antisense oligonucleotides (ASOs) and microRNAs (miRNAs).

Antisense oligonucleotides (ASOs)

ASOs are typically 18–20 bp strands that bind to target (pre-) mRNA and modify expression by either altering splicing, recruiting RNAseH for target cleavage and degradation, inducing translational arrest, inhibiting RNA-binding, or increasing translational activity.[190] Depending on the specific mutation, different ASO strategies can be implemented.

The most successful ASO thus far in the neurodegenerative field, is nusinersen (Spinraza) and its newer analogs that target splicing of the SMN1 gene for the treatment of spinal muscular atrophy type 1 (SMA1), a devastating pediatric neurological disorder.[186,187] Nusinersen and its analogs are splice-modifying ASOs. In this mechanism, the ASO binds to a splice site at the intron–exon junction and disrupts splicing of pre-mRNA, inducing exon skipping. This strategy has recently been applied to REDs, for example, in SCA3[191] and Duchenne Muscular Dystrophy,[192,193] in the latter case advancing to clinical trial.

To target putative gain-of-function toxicity induced by polyQ expansions, the favored ASO strategy for REDs has been induction of gene knockdown through RNAseH recruitment. It is worth mentioning that validation of this strategy is often based on overexpression or knock-in models. The former presents a somewhat circular logic wherein toxicity is induced by overexpression of a specific gene and then reversed by knockdown of that same gene. These models do allow for analyzing rescue by targeting alternative pathways and still have great utility. Knock-in mouse models, while a better replica of human physiology, often employ nonhuman sequences, thereby making target engagement difficult to evaluate. Safety of knockdown has been largely predicated on knockout mouse models, but interpretation can be colored by the chosen phenotype and may not be predictive over the life span of a mouse. For example, the *ATXN3* knockout mouse was largely behaviorally normal, but did show increased ubiquitination in brainstem and peripheral tissue slices.[194] *HTT* knockout mice had no neurological phenotype, but did develop acute pancreatitis, suggesting tissue-specific roles for these proteins. In a CNS-specific ASO application, reducing *HTT* expression was not toxic in mice, nonhuman primate studies, or in early clinical trials.[195] These types of data are crucial as most knockdown ASO strategies are not allele-specific and will thus reduce expression of *both* WT and expanded alleles (effectively recreating a knockout phenotype). There have been efforts to knockdown strictly the mutant allele, taking advantage of single-nucleotide polymorphisms (SNPs) that may be present on the mutant allele only. This strategy depends on the presence of the specific targeted SNP and is thus not as broadly applicable.

In HD, both allele-nonspecific[195] (Clinical trial NCT03342053) and allele-specific (Clinical trial NCT03225833) ASOs entered into clinical trials. In SCA3, there is a potential promising candidate for allele-nonspecific ASO-mediated therapeutic knockdown,[119,196,197] as well as an ASO targeted against the CAG repeat mutation itself.[198] Two Phase I clinical trials for an ASO against the *C9ORF72* expansion in ALS are also underway (Clinical trials NCT03626012 and NCT04288856). At the time of writing this text, both ASO trials for HD were halted prematurely. While we await more complete data and analysis, early indications are of limited efficacy or target engagement rather than concern with safety. In one case, Tominersen (formerly HTT_{Rx}), the target was engaged, demonstrated by reduction of *HTT* protein levels in patient CSF[195] in the phase II trial. The allele-specific ASO, WVE-120102 (Clinical Trial NCT03225833), did not show as significant a reduction in HTT levels as Tominersen, but it is possible that dosing may be able to overcome this hurdle. We return to the failure of these trials below.

miRNAs

miRNAs are endogenous short RNAs that imperfectly complement sites within transcripts (typically within the 3′UTR) and downregulate gene expression via suppressing translation, stimulating deadenylation and degradation, or inducing target cleavage.[199] miRNAs were first shown to regulate CAG repeat expansion expression in SCA1.[200] The ATXN1 gene has a long 3′UTR and is under regulation by multiple miRNAs, a few of which were shown to reduce polyQ ATXN1 expression. This approach is being investigated for other SCAs also: the miRNA *bantam* decreases polyQ ATXN3 toxicity in a *Drosophila* model of SCA3[201] and miR-146a reduces levels of TATA-binding protein (TBP), the gene expanded in SCA17.[202]

Global profiling of miRNAs in REDs has shown that they likely play a role in pathogenesis. Further, miRNAs are often tissue- or cell-type-specific and thus may contribute to selective cellular vulnerability. For example, miRNA profiling in lymphoid and iPS-derived neural tissues from SCA3 patients was vastly different, even when sourced from the same patient.[203,204]

Thus, miRNAs represent another genetic approach to treating REDs, either by inducing expression of endogenous miRNAs that reduce expression of the expanded gene or by exogenous delivery. The ability of miRNAs to affect both RNA and protein levels adds to their attractiveness as a therapeutic platform.

Small molecules targeting RNAs with repeat expansions

RNAs harboring either a CAG or a CTG repeat expansion can also be targeted by small molecules.[205–208] In some cases, these molecules interact with the A-A mismatch that forms with repeat-associated hairpin RNAs.[209] Thus, secondary RNA structure is likely a key determinant in the success of these molecules and may vary pending repeat type (e.g., CAG vs. CTG). Repeat location also affects response to these compounds. For example, a compound designed against CTG repeats (specifically in FECD and DM1) had differential effects pending repeat location. In FECD (intronic repeat), the molecule led to decay via the exosome, whereas it blocked sequestration of RBPs in DM1 (3′UTR repeat).

While early work, the idea of targeting repeat expansions with nongenetic (e.g., small molecule) therapies opens the door for alternative approaches with very attractive qualities: (1) targeting the diseased gene, (2) affecting only the expanded allele, and (3) potentially improved systemic distribution as opposed to the local spread from an injected ASO.

Future of RED translational medicine

The last decade has seen our understanding of REDs grow exponentially. Historical development of therapeutic treatment for neurodegenerative diseases has been challenging, with many clinical trials failing to reach the desired disease-halting clinical outcomes. The recent failure of the HD clinical trials will undoubtedly provoke soul searching among not only the RED community, but the neurodegeneration community in general. In Table 18.2, we propose several possibilities and offer suggestions on how to guide the development of both nucleotide and nonnucleotide-targeting therapeutics. Firstly, hypotheses regarding the target may be incorrect. It could be that in humans, the disease is not as straightforward as a simple toxic gain of function, but rather by complex cellular changes as a consequence to polyQ expansion, which we discussed above. Some of these consequences, if loss of function, may even be exacerbated by knocking down the target protein, especially with strategies that do not discriminate between the pathologic and nonpathologic allele. Patient-derived iPSCs and differentiated neurons allow us to better study on- and off-target effects, whether genetic or small-molecule therapies, even with limited clinical samples.[210,211] Although not without its caveats as a technology, differentiating multiple neuronal and glial subpopulations from patient-derived iPSCs now puts a mechanistic understanding of the *human* biology and cell type specificity of these diseases within reach. Patient-specific cell cultures also provide an invaluable model system by which we can identify additional targets and test both genetic and small-molecule therapies for on and off-target effects. Finally, genome editing, including of repeat expansions, now enables systematic comparison of knockdown with the theoretical gold standard of genetic correction.[212]

Second, it could also be that by the time the disease manifests (and it should be emphasized that the trials to date have focused on patients with established disease phenotypes), the genetic or pharmacological knockdown approaches may no longer be sufficient as monotherapy. RED pathology reaches beyond the nucleotide level (Fig. 18.2), suggesting that genetic therapies may greatly benefit when combined with those targeting downstream pathways for maximum efficacy. There may be downstream consequences within the cell—for example, widespread altered proteostasis and protein aggregation beyond the mutated protein, consistent with the prion hypothesis.[102] Combination therapies, for example, an antisense approach with an autophagic inducer or other modulator of proteostasis, may be beneficial. Or if ion channels or synaptic transmission is disrupted early in disease pathogenesis, then therapeutics intervening on those levels, together with genetic intervention to correct the transcriptional mutation, will be complementary. There also may be key non-cell-autonomous pathologies by the time disease is established, for example, neuroinflammation, such that correcting the levels of the mutated protein may not be beneficial on its own, as the downstream damaging signaling cascade has already been activated. ASO approaches (as monotherapy) may thus be better suited to patients in the presymptomatic/prodromal phases of disease. It would likely also be more effective to intervene in patient populations that are as clinically homogeneous as possible.

Lastly, as we look to translating benchtop research to bedside therapies, it behooves us to return to the clinical phenotype and how we measure patient disease progression in clinical trials. Thus far, most trials center their primary outcome on a clinical score for the primary phenomenology (e.g., Scale for Assessment and Rating of Ataxia [SARA] for ataxia or the Unified Parkinson Disease Rating Scale [UPDRS]-motor for PD). However, these

TABLE 18.2 Pitfalls and proposed solutions.

Pitfall	Proposed studies or redesign
Genetic knockdown achieves a distinct genotype from wild type, rendering comparisons difficult to interpret.	Employing iPSC models with genomic editing to create isogenic lines with varying repeat lengths for systematic cross-comparison.
Patients with advanced disease may be beyond the aid of current therapeutics.	Pivot trial recruitment to prodromal or presymptomatic patients to examine preventative qualities of therapeutics.
Patient population is too heterogeneous	Condense trial enrollment to a smaller, better characterized population.
Interexaminer variability of clinical scales prevents objective tracking of patient progression.	Utilize objective biomarkers and biometrics. Model the natural history to measure treatment-induced shifts in progression curves.

metrics, while undoubtedly valuable in tracking change in the clinic, lack sensitivity and are limited due to interexaminer variability.[213,214] Further, REDs are systemic mutations with wide-reaching effects (Fig. 18.4). Thus, we must consider how peripheral issues progress and are affected by therapeutics. Collectively these difficulties point to the need for improved objective biomarkers to monitor disease progression and track target engagement. While there have been advances in structural and functional imaging of the CNS with longitudinal volumetric analyses,[215,216] there remains a void outside the CNS. For example, NCS in SCA2 or SCA3 or retinal tomography in SCA7 might provide noninvasive measures for research and also yield insights into clinical symptoms. Early diagnosis and progression markers will be key to successful therapeutic advancement.

Key questions for future research

- CAUSE: In REDs, beyond the initial genetic lesion, what is the central driver of pathology? Alterations at the genetic, epigenetic, RNA, and protein level have been implicated, but what is their relative contribution in different diseases?
- VULNERABILITY versus PROGRESSION: Is the driver of *vulnerability* in REDs the same as the driver of *progression*? Do we need to do more thorough human genetic studies to identify genetic modifiers of *progression* rather than cause?
- MODELS: What are the best cellular and animal models for REDs? Do we need different models to better capture vulnerability and progression in REDs? How does the relative ease and tractability of cellular and simple animal models trade-off against what could be complex effects during human development and aging of repeat expansions? Are epigenetic changes, aging, and complex neuroglial interactions required for emerging human stem-cell models of REDs to be effective models of disease?
- THERAPEUTIC TARGET: In REDs, whether at the genetic or cellular level, is the point of initiation the appropriate target of intervention? For example, is the repeat expansion itself a viable therapeutic target or should we target a downstream cellular consequence? Is it sufficient to correct perturbed neuronal pathology or is it necessary to consider effects on other cells and body systems, including glial and neuroinflammatory changes?
- COMBINATION THERAPIES: Does the appropriate target change at different stages of the disease, and by consequence, will we need combination therapies to most comprehensively correct a disease that could be at different stages in different cell types?
- BIOMARKERS and CLINICAL TRIALS: How do we track target engagement and which patient population is best served? How do we optimize dosing and delivery of genetic therapies? What objective metrics can we incorporate to better inform us of patient diagnosis to facilitate early intervention or to measure patient outcomes to experimental therapeutics? Are diagnostic biomarkers the same as clinical progression markers?

Conclusion

Since the discovery that CGG repeats at the *FMR1* locus in FXS,[217,218] the repeat expansion universe has blossomed. The field has been distinguished not only for the insights into molecular genetics and cell biology of RED disorders, but for providing principles that have led to the understanding of the commonest neurodegenerative disorders.[219] An emerging field has even tied the REDs to these more common disorders.[52,53,220] Here, just scraping the surface, we hope we have conveyed the richness and the complexity of these diseases from their biological underpinnings to their clinical manifestations. Just as the REDs ushered in a revolution in human genetics forever altering our understanding of neurodegeneration, we can only hope that these same disorders raise depths of understanding neurologic and nonneurologic manifestations of disease, better diagnostics and biomarkers and, of most concern to our patients, an era of specific disease-modifying therapies.

Acknowledgments

Figures were made with the assistance of BioRender Software.

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