

Unstable Repeat Expansion in Neurodegenerative Dementias: Mechanisms of Disease

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Received: February 17, 2011
Revision received: March 7, 2011
Accepted: March 7, 2011

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The majority of neurodegenerative dementias are thought to result primarily from the misfolding, aggregation and accumulation of proteins which interfere with protein homeostasis in the brain. Some of them are caused by the expansion of unstable nucleotide repeats, which include Huntington's disease as a prototype. Other neurodevelopmental or neurodegenerative disorders, such as fragile X syndrome, some spinocerebellar ataxias and myotonic dystrophies exhibit cognitive or behavioral deficits as parts of their clinical manifestations. Unstable repeat expansions include trinucleotide, tetranucleotide, and pentanucleotide. Recently hexanucleotide repeat expansion in frontotemporal dementia and amyotrophic lateral sclerosis was identified. The pathogenic mechanisms for these repeat disorders include either loss of protein function or gain of function at the protein or RNA levels. The aim of this article is to review proposed mechanisms by which unstable repeat expansions give rise to degeneration of brain with the hope of understanding the diseases and providing insights into the areas of therapeutic intervention. We will review these potential mechanisms in the context of fragile X syndrome, Huntington's disease, spinocerebellar ataxias, myotonic dystrophy, and frontotemporal dementia and amyotrophic lateral sclerosis. We will also discuss the potential targets for therapeutic intervention.

Key Words: *Dementia, Unstable repeat expansion*

INTRODUCTION

Protein misfolding and aggregation is the most likely cause of various neurodegenerative dementias [1]. This diverse group includes Alzheimer's disease (AD), dementia with Lewy bodies (DLB), Parkinson's disease with dementia (PDD), multiple system atrophies (MSAs), frontotemporal lobar degeneration (FTLD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), prion diseases and Huntington's disease (HD). These neurodegenerative dementias can be broadly divided into categories based on particular molecular profiles: β -amyloidopathies, α -synucleinopathies, tauopathies, prionopathies and polyglutaminopathies.

The distinguished pathogenic mechanism in HD among other common neurodegenerative dementias emphasizes the importance of unstable repeat expansion disorders in the pathogenesis of dementias (Table 1). Keeping step with the recent identification of hexanucleotide repeat expansions in

frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), we expect this timely review should expedite our understanding of neurodegenerative dementias beyond the current concept of dementias caused by protein misfolding disorder.

Among the diseases mentioned above, HD is one of the polyglutamine disease. Polyglutamine diseases or polyglutaminopathies constitute a class of nine genetically distinct neurological disorders that are caused by expansion of CAG repeats which produce pathogenic proteins containing expanded tracts of glutamines [2]. These disorders include HD, dentatorubral-pallidoluysian atrophy (DRPLA), spinal and bulbar muscular atrophy (SBMA, also known as Kennedy's disease) and spinocerebellar ataxia 1, 2, 3, 6, 7, and 17 (SCA1/2/3/6/7/17). They are autosomal dominant except for SBMA which is X-linked.

For most CAG repeat expansions in the coding region of a gene, the function of the affected proteins remains unknown.

Table 1. Genetics and clinical features of unstable repeat expansion disorders causing dementia

Disease	Inheritance	Loci on chromosome	Repeat units	Main clinical features other than dementia
Trinucleotide repeat disorders				
FRAXA	XL	Xq27	(CGG) _n	Macroorchidism, connective tissue dysplasia
FXTAS	XL	Xq27	(CGG) _n	Tremor, ataxia, parkinsonism
HD	AD	4p16	(CAG) _n	Chorea, dystonia
DRPLA	AD	12p13	(CAG) _n	Ataxia, chorea, myoclonus, epilepsy
SCA17	AD	6q27	(CAG) _n	Ataxia, dysarthria, epilepsy, hyperreflexia
DM1	AD	19q13	(CTG) _n	Myotonia, weakness, cardiac abnormalities, testicular atrophy, insulin resistance, cataracts
Tetranucleotide repeat disorder				
DM2	AD	3q21	(CCTG) _n	Similar to DM1, no congenital form
Pentanucleotide repeat disorder				
SCA10	AD	22q13	(ATTCT) _n	Ataxia, dysarthria, nystagmus, epilepsy
Hexanucleotide repeat disorder				
c9FTD/ALS	AD	9p21	(GGGGCC) _n	Spasticity, weakness, muscle wasting

AD, autosomal dominant; c9FTD/ALS, chromosome 9 frontotemporal dementia and amyotrophic lateral sclerosis; C9ORF72, chromosome 9 open reading frame 72; DM1, dystrophia myotonica type 1; DM2, dystrophia myotonica type 2; DRPLA, dentatorubral-pallidoluysian atrophy; FRAXA, fragile X syndrome; FXTAS, fragile X-associated tremor/ataxia syndrome; HD, Huntington's disease; XL, X-linked; ORF, open reading frame; SCA10, spinocerebellar ataxia type 10; SCA17, spinocerebellar ataxia type 17.

In each of these diseases, the mutant protein and the distribution of regions with neuronal degenerations are different. However, all of these diseases have a common underlying pathogenic mechanism. It is assumed that the pathogenesis may be directly linked to the expanded polyglutamine tracts [2].

Although the mechanisms of unstable repeat expansions are unclear, dynamic mutation has been suggested as a plausible mechanism. Dynamic mutations refer to differences in repeat-DNA copy number, distinguishable from other types of mutations [3]. Dynamic mutations are suggested as the molecular basis of trinucleotide repeat expansions. However, this form of mutation is not restricted to trinucleotide repeats. At each dynamic mutation locus, there is a normal range of repeat-sequence copy numbers from 3 to 33 bp above which the repeats become unstable [4]. As a result "DNA slippage" occurs, which refers to loop out structures forming during DNA replication because of the repeat-sequence in the DNA, while complementary base-pairing is maintained between the parent and the corresponding daughter strands. If the DNA slippage occurs on the daughter strand, this results in increased repeat numbers. When the number of copies increases beyond a crucial threshold length, the repeats surpass the capacity of genomic safeguard and manifest in diseases. Generally, the larger the repeat expansions are, the more likely to give rise to diseases or increase the disease severity these are. This property accounts for premutation or carrier allele, founder effect, and anticipation [4]. In premutation, the length

of repeats is below a threshold, but individuals with a premutation allele exhibiting a normal phenotype have increased susceptibility to mutation in a subsequent transmission resulting in subsequent founder effects. Anticipation is the propensity of repeats above a threshold to increase through successive generations, hastening the onset age and increasing disease severity.

The length of repeats has different effects on the stability and the transmission through generations depending on whether the repeats are located in the coding sequences or in non-coding regions such as intron, 5' untranslated region (UTR), or 3'UTR. For repeats in the coding sequences, the repeats become unstable at approximately 30 units and the changes in tract size are 10 repeats or less per generation. On the contrary, repeats in the non-coding regions become unstable at longer units than those in the exons, and the parent to child transmission increases by 100-10,000 units per generation [5, 6].

1. Various nucleotide repeat units and phenotypes

The list of unstable repeat expansion disorders has increased to include not only trinucleotide repeats but also tetra- [7], penta- [8] and hexa-nucleotide repeats [9, 10]. In all these diseases, disease-specific phenotypes are determined by numbers of the expanded repeats, their subcellular localization, and the interactions of the proteins and RNAs affected. The unstable repeat expansions result in neurodevelopmental or

neurodegenerative disorders depending on the onset age of the diseases. However, the boundary between two disorders is quite often ambiguous. From a cognitive and behavioral perspective, the repeat expansions can give rise to neurodevelopmental disorders exhibiting mental retardation or neurodegenerative disorders showing dementia or a combination of both. In case of fragile X syndrome spectrum which is X-linked, expansions within a single fragile X mental retardation 1 gene (*FMRI*) can result in either a neurodevelopmental disorder by loss of function disorder at the level of protein or a neurodegenerative disorder through gain of function disorder at the level of RNA [11, 12]. The number of CGG repeats more than 200 in *FMRI* give rise to typical fragile X syndrome (FRAXA) which is a neurodevelopmental disorder as one of the common forms of mental retardation, whereas the repeat number of 60-200 causes a clinically distinct syndrome, fragile X-associated tremor/ataxia syndrome (FX-TAS) which is a neurodegenerative disorder affecting older males [11, 12]. In this context, FRAXA is included in this review to explain the loss of protein function mechanism.

The myotonic dystrophies are multisystem disorders which are caused by trinucleotide and tetranucleotide repeat expansions [7, 13]. Dystrophia myotonica 1 (DM1) is known to be an adult-onset muscular dystrophy caused by an expanded CTG trinucleotide repeats in the 3' UTR of the dystrophia myotonica protein kinase (*DMPK*), whereas dystrophia myotonica 2 (DM2) is caused by a CCTG tetranucleotide expansion in intron 1 of the zinc-finger protein 9 (*ZNF9*) [14]. When compared to muscle and cardiac manifestations, the understanding of neurological aspects, such as cognitive impairment and personality changes of DM1 [15] has been overlooked in the past. It has been shown that splicing patterns of several genes such as the *N*-methyl-*D*-aspartate (NMDA) receptor NR1 subunit, the neuronal microtubule-associated protein Tau, and the amyloid precursor protein (APP) transcript are also altered in the brain of the patients with DM1 [16].

Spinocerebellar ataxias (SCA) are genetic nomenclature for autosomal dominant cerebellar ataxias caused by degeneration of the spinal cord and cerebellum and its afferent and efferent connections [17]. SCA are highly heterogeneous and the prevalence of specific subtypes varies with different ethnic backgrounds. Out of thirty six subtype of SCA identified

thus far [18], seven subtypes of polyglutamine diseases (SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, and DRPLA) belong to this category. Among them, DRPLA and SCA17 are of interest to get attention because these have dementia as one of their cardinal features, in addition to other neurological manifestations, resembling symptoms of HD [17, 19, 20]. However, executive dysfunction in SCA1 and mild verbal memory deficits in SCA1, SCA2, and SCA3 were found, but rarely progresses to gross dementia [21]. Spinocerebellar ataxia type 10 (SCA10) is caused by an unstable expansion of the ATTCT pentanucleotide repeat in intron 9 [8, 22]. SCA10 is characterized by ataxia and seizures, in company with cognitive and behavioral deficits varying from mild cognitive impairment to dementia [23, 24].

Frontotemporal lobar degeneration (FTLD) is a class of neurodegenerative disorders associated with focal degeneration, with the predilection for frontal and temporal multimodal association cortices. Pathologically, FTLD can be classified according to the major proteins present in inclusion bodies: tau (FTLD-tau), transactive response DNA-binding protein (TARDBP or TDP-43) (FTLD-TDP) and fused in sarcoma (FUS) (FTLD-FUS) [25]. TDP-43 pathology is also pathological characteristics of MND, along with superoxide dismutase 1 (SOD1), FUS, optineurin (OPTN) and valosin-containing protein (VCP) [26].

FTD and ALS were thought to be independent diseases until the frequent co-occurrence of these two disorders was recently demonstrated, suggesting that they may share a potential common etiological pathway [27]. Two independent groups have recently reported an expanded GGGGCC hexanucleotide repeat in non-coding region of chromosome 9 open reading frame 72 (*C9ORF72*) as the most common mutation in FTD/ALS families with combined TDP-43 pathology [9, 10].

Several pathogenic mechanisms have been proposed in diverse classes of unstable repeat expansion disorders: diseases that are caused by expansions of non-coding repeats resulting in loss of protein function; those that are caused by expansions of coding repeats resulting in altered protein function; and a class of diseases that are caused by expansions of non-coding repeats giving rise to dysfunction at RNA level [28]. For the group of diseases that are caused by a loss of protein

function mechanism, the normal functions of the disease proteins are central to the underlying pathogenesis and clinical phenotypes. In polyglutamine diseases, the same toxic moiety is shared among nine different disease proteins, but phenotypes of these diseases are clinically distinct. Differences are determined not only by the repeat length, but also by the intrinsic function of the causative proteins. Finally, in diseases caused by expanded non-coding repeats that are mediated at the RNA level, there is emerging evidence that pathogenic RNA species induce aberrant interactions of RNA and protein, leading to neuronal dysfunction and loss (Table 2).

2. Diseases caused by loss of normal protein function

FRAXA is associated with an array of cognitive and behavioral problems including learning deficits. Most cases are caused by expansion of a CGG trinucleotide repeat in the 5' UTR of the fragile X mental retardation 1 gene (*FMRI*), resulting in transcriptional silencing and functional loss of the protein FMRI, FMRP. Loss of FMRP has widespread effects interfering with neuronal development and resulting in cognitive deficits. Full mutation with tracts of more than 200 CGG repeats give rise to FRAXA, whereas a premutation with 60 to 200 repeats results in a clinically distinct syndrome, fragile X tremor/ataxia syndrome (FXTAS) [11].

FMRP is cytoplasmic protein and abundant in neurons. It is a RNA-binding protein which plays a role in translational regulation of its RNA targets [29]. In dendritic spines, its activity is upregulated in response to stimulation by metabotropic glutamate receptors (mGluRs) [30]. Stimulation of mGluRs also results in internalization of α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, which is important in the expression of long-term depression (LTD). *Fmr1*^{-/-} mice show behavioral abnormalities and selective enhancement of mGluR-dependent LTD [31]. Reduced or absent FMRP results in over-amplification of the mGluR response and increased LTD. Such alterations in activity-dependent synaptic plasticity could underlie cognitive deficits in FRAXA. This is strongly supported by the study which used a *Drosophila* model of the disease, showing rescue of neuronal and behavioral phenotypes with mGluR antagonists [32].

Furthermore, animal models of FRAXA using *FMRI* knockout mice exhibited deficits in learning and memory, similar to the human phenotypes [33, 34].

3. Diseases caused by gain of function at the protein level

Among the nine polyglutamine diseases, the disease-causing proteins are expressed widely in the CNS, but specific

Table 2. Molecular features of unstable repeat expansion disorders with dementia

Disease	Repeat units	Gene (protein)	Location of repeat in gene	Putative function	Normal repeat length	Pathogenic repeat length
Disease caused by loss of normal protein function						
FRAXA	(CGG) _n	<i>FMRI</i> (FMRP)	5'UTR	Translational regulation	6-60	> 200 (full mutation)
Diseases caused by altered protein function						
HD	(CAG) _n	<i>HD</i> (HTT)	Coding region	Transport, transcription	11-34	38-121
DRPLA	(CAG) _n	<i>DRPLA</i> (atrophin 1)	Coding region	Transcription	3-36	49-88
SCA17	(CAG) _n	<i>SCA17</i> (TBP)	Coding region	Transcription	29-42	47-55
Diseases caused by alteration at RNA level						
DM1	(CTG) _n	<i>DMPK</i> (DMPK)	3'UTR	RNA-mediated	3-37	50-1,000
DM2	(CCTG) _n	<i>ZNF9</i> (ZNF9)	Intron 1	RNA-mediated	10-26	75-11,000
FXTAS	(CGG) _n	<i>FMRI</i> (FMRP)	5'UTR	RNA-mediated	6-60	60-200 (premutation)
SCA10	(ATTCT) _n	<i>ATXN10</i> (ataxin 10)	Intron 9	RNA-mediated	10-29	550-4,500
Disease of still unknown pathogenic mechanism						
c9FTD/ALS	(GGGGCC) _n	<i>C9ORF72</i> (C9ORF72)	Promoter or intron 1	Unknown	23	700-1,600

AD, autosomal dominant; c9FTD/ALS, chromosome 9 frontotemporal dementia and amyotrophic lateral sclerosis; ATXN10, ataxin10; C9ORF72, chromosome 9 open reading frame 72; DM1, dystrophia myotonica type 1; DM2, dystrophia myotonica type 2; DMPK, dystrophia myotonica protein kinase; DRPLA, dentatorubral-pallidolulysian atrophy; DM, dystrophia myotonica; FMRP, fragile X mental retardation protein; FMRI, fragile X mental retardation 1; HD, Huntington's disease; HTT, huntingtin; XL, X-linked; ORF, open reading frame; SCA10, spinocerebellar ataxia type 10; SCA17, spinocerebellar ataxia type 17. TBP, TATA box binding protein; 3'UTR, 3' untranslated region; 5'UTR, 5' untranslated region ZNF9, zinc-finger protein 9.

groups of neurons are selectively vulnerable in each disease, resulting in characteristic patterns of neuronal degeneration and phenotypes [2]. Genetic data in human have established that these diseases result from a gain of function mechanisms [2]. Accumulation of the mutant proteins into insoluble aggregates is a common feature of polyglutamine diseases. Aggregates are believed to be sequestered in inclusions which also include molecular chaperones and impair the ubiquitin proteasome system (UPS) [35]. These also recruit normal cellular proteins through aberrant protein-protein interactions leading to neuronal degeneration [36].

1) Huntington's disease (HD)

We will first explain this gain of toxic function with HD which has the highest prevalence among CAG repeat disorders and present with subcortical dementia along with chorea. Huntingtin (HTT) is a large cytoplasmic protein found associated with synaptic vesicles and microtubules. Patients who are homozygous for the mutant gene are neurologically normal until the onset of the disease, suggesting that the expanded polyglutamine tract does not cause the loss of the normal protein function, but the novel gain of function of the mutant protein.

It has been shown that a number of proteins interact with HTT (HTT-interacting proteins), such as HTT-interacting protein 1 (HIP1) [37, 38], Src homology region 3-containing Grb2-like protein 3 (SH3GL3) [39], protein kinase C and casein kinase substrate in neurons 1 (PACSIN1) [40], HTT-associated protein 1 (HAP1) [41], postsynaptic density-95 (PSD-95) [42], specificity protein 1 (Sp1) [43, 44] and nuclear receptor co-repressor (N-CoR) [45, 46]. These interactions change in the pathologic conditions in which, for instance, the interactions of HTT with HAP1 are increased and the interactions with HIP1 are decreased. If mutant HTT has a decreased binding for HIP1, this may result in toxicity mediated by increased intracellular HIP1 which is neurotoxic when over-expressed [47]. Although the precise roles of these HTT-interacting proteins remain unknown, their putative roles raise the possibility that changes in the interactions may contribute to the pathogenesis of the disease.

The neurons in the striatum (caudate nucleus and putamen) are sensitive to glutamate and the medium-sized spiny GAB-

Aergic neurons of the striatum degenerate first in early HD, resulting in atrophy of the caudate and putamen which is the pathological hallmark of HD. The striatal neurons are also the main target for nigrostriatal dopaminergic input and dopamine modulates the glutamate toxicity [48]. Thus, a change in the activity of glutamate input could increase the vulnerability of striatal neurons.

In HD, neuronal intranuclear deposits, also known as neuronal intranuclear inclusions (NIIs), of a mutant HTT are a key feature [49]. Although there has been much debate regarding the role of these NIIs, particularly whether or not they are neurotoxic or neuroprotective, it seems highly likely that they play a role in polyglutamine toxicity [50].

2) Dentatorubral-pallidoluyian atrophy (DRPLA)

DRPLA is characterized by dementia, ataxia, chorea, myoclonus, and epilepsy [19]. It is caused by expanded CAG repeats in *DRPLA* encoding atrophin 1 on chromosome 12p13, which results in a polyglutamine tracts leading to a toxic gain of function [51, 52]. The atrophin 1 protein is expressed widely in the brains. The function of atrophin 1 remains unknown, however, is believed to be a transcriptional co-repressor [53]. In DRPLA, NIIs containing mutant atrophin 1 are found in the degenerated neurons mainly in the dentate nucleus of the cerebellum, red nucleus, globus pallidus, and subthalamic nucleus. It has been found that the cleavage product of atrophin 1 may be relevant to pathogenesis leading to abnormal nuclear interactions and cell toxicity, whereas full-length of atrophin 1 may not be toxic [53, 54].

3) Spinocerebellar ataxia type 17 (SCA17)

SCA17 which has phenotypes similar to DRPLA, is caused by an abnormal expansion of CAG repeats in the TATA-binding protein gene (TBP) which is a general transcription initiation factor [55]. A mouse model for SCA17 showed that the mutant TBP interacts with transcription factor IIB, preventing it from regulating the expression of its target gene, the small heat shock protein 1 (HSPB1) which is a potent neuroprotective factor. Furthermore, over-expression of TFIIB and HSPB1 rescued neurodegeneration [56]. It has also been shown that the mutant TBP in transgenic mouse binds to promoter DNA [57].

4. Diseases caused by alteration at RNA level

1) Dystrophia myotonica (DM)

Among hypotheses proposed to explain the pathogenesis of DM1, RNA-mediated pathogenesis has widely been accepted to be a major mechanism [58]. There is striking clinical overlap between DM1 and DM2, despite the mutations in different genes on separate chromosomes and in the context of unrelated neighboring genes. So a common pathogenic mechanism has been suggested that involves aberrant interactions of expanded RNAs with general RNA-binding proteins (RBPs), with subsequent protein dysfunction. The general idea is that mutant expanded RNAs sequester RBPs, thereby affecting alternative splicing of their target RNAs. This aberrant alternative splicing underlies the pathogenic phenotypes of DMs.

However, other hypotheses about the pathogenesis have been proposed. Studies using *Dmpk*^{-/-} mice argued against the *DMPK* haploinsufficiency hypothesis [59, 60]. These mice developed only mild myopathy and did not show other manifestations of DM1. Study of mice deficient in sine oculis-related homeobox 5 homologue (*Six5*) which is a transcription factor downstream of *DMPK* failed to support the hypothesis that the effects of the expanded allele on the neighboring genes cause the phenotypes [61].

Based on RNA-mediated pathogenesis, RBPs regulate splicing, and abnormal splicing of specific pre-mRNA targets is thought to be accountable for the various phenotypes in DM. Thus far two families of RBPs have been implicated in the pathogenesis of DM. CUG repeat RNA-binding protein 1 (CUG-BP1) and ETR3-like factors (CELFs), and muscle-blind-like (MBNL) proteins have the ability to interact with RNAs containing CUG or CCUG repeats [62, 63]. Expanded CUG repeats in mutant DMPK protein form nuclear RNA foci that recruit MBNL proteins. An *Mbnl* knockout mouse model develops DM-like phenotypes and shows dysregulation of alternative splicing in the pre-mRNA of skeletal muscle chloride channels [64]. The function of CUG-BP1 is increased in DM, although it is not recruited to the ribonuclear inclusions [65]. Mouse models of CUG-BP1 over-expression develop muscle phenotypes with characteristic DM splicing alterations [66]. Many of the splicing alterations indicate that

CELF and MBNL proteins might function antagonistically to regulate common pre-mRNA targets. When it comes to the brain, among 13 pre-mRNA targets which have been shown to be altered in DM, the NMDA receptor NR1 subunit, the neuronal MAPT, and the APP transcript were found implicated in the CNS [16].

Some key experiments support this RNA-mediated pathogenesis in DM [67]. The nuclear RNA foci of the affected cells include MBNL, whose binding to the CTG repeats are augmented with expanded repeats [63, 68]. DM1 and DM2 have the mutations in different genes on separate chromosomes, but show similar phenotypes [7]. The expression of mutant repeats in a non-coding region of another gene give rise to the phenotypes similar to those seen in DM [69, 70]. The deletion of MBNL reproduces the phenotypes of DM1 [64]. Furthermore, over-expression of MBNL rescues some of DM phenotypes [71]. Altered MBNL-mediated splicing of RNAs is found in DM tissue [68].

2) Fragile X-associated tremor/ataxia syndrome (FXTAS)

In contrast to full mutation which causes decreased levels of FMRP, several findings have shown that the premutation with 60-200 repeats does not result in decreased FMRP levels but leads to the 2-5 fold increase in *FMRI* mRNA [72-74]. These findings suggest RNA-mediated pathogenesis for FXTAS. It has been reported that elderly men with FXTAS had elevated level of *FMRI* mRNA and the premutation and normal or borderline FMRP levels supporting RNA-mediated pathogenesis [11]. A knockin mouse in the *FMRI* gene with 100 CGG repeats showed increased levels of *FMRI* mRNA and neuronal intranuclear inclusions in the presence of normal FMRP levels [75]. Furthermore, over-expression of CGG repeats in *Drosophila melanogaster*, which is transcribed but not translated, was sufficient to cause inclusions and phenotypes [76].

3) Spinocerebellar ataxia type 10 (SCA10)

It was identified that SCA 10 is caused by a large expansion of an intronic ATTCT repeats in the *ATXN10* gene on chromosome 22 [8]. The *ATXN10* gene encodes ataxin 10, which contains two armadillo repeats that are known to mediate protein-protein interaction. Although ataxin 10 is considered

to play a role in neuronal survival and differentiation, the exact function remains unknown. After subsequent studies which showed that neither a gain in function nor a toxic function of ataxin 10 is the major pathogenic mechanism, but rather, the expanded repeats co-localized with heterogeneous nuclear ribonucleoprotein K (hnRNP K) which is diminished in SCA10 cells and transgenic mice [77]. The hnRNP K is one of RBPs and influences pre-mRNA processing and other aspects of mRNA metabolism and transport. Cells expressing the expanded repeats underwent apoptosis. Importantly, either blocking the expanded repeats or over-expressing hnRNP K rescued cells from the SCA10 mutation-induced death, indicating that the mutant RNA inactivates hnRNP K through RNA-protein interaction and causes neuronal death. The mechanism for SCA10 is believed to be RNA-mediated by the expanded repeats in a mutant RNA [77, 78].

5. Diseases of still unknown pathogenic mechanism

There is a pathologic overlap between FTD and amyotrophic lateral sclerosis (ALS) suggesting that they are part of a disease spectrum of TDP-43 proteinopathies [79, 80]. Moreover, abnormal subcellular localization and aggregates of TDP-43 are found in most patients with FTD and ALS. It is strongly supported by evidence showing the existence of family members affected by FTD, ALS or a mix referred to as FTD-MND. The genetic basis for this association of FTD and ALS was discovered to be an expansion mutation of GGGGCC hexanucleotide in a non-coding region of *C9ORF72*, referred to now as c9FTD/ALS [9, 10]. Hexanucleotide repeats reside in the intron between non-coding exons. Wild-type alleles contain no more than 23 repeats, compared to mutant alleles having approximately 700 to 1600.

At this point, the functions of the *C9ORF72* protein remain unknown. The location of these repeats within the intron suggests a possible role of non-coding repeat expansion disorders. The role of the pathogenic RNA repeats could be a strong candidate for development of the disease as in DM. Notably it was found that the repeat expansion leads to the loss of one alternatively spliced *C9ORF72* transcript suggesting that the loss of one of at least three transcripts may have a significant impact on selective cell types [9]. It was also shown that the

expanded repeats lead to formation of nuclear RNA foci [9]. These findings suggest that multiple potential pathogenic mechanisms may be associated with this repeat expansion including a direct effect on the expression of *C9ORF72* by affected transcription and an RNA-mediated gain of function mechanism through the formation of toxic RNA foci. It would be crucial to identify RNA-binding proteins to which *C9ORF72* RNA transcripts bind and understand the effect of this binding on the alternative splicing of *C9ORF72* transcript. Considering that TDP-43 pathology is a feature of c9FTD/ALS and TDP-43 is one of nuclear RNA-binding proteins, it is possible that TDP-43 may bind the *C9ORF72* transcript. However, this interaction seems quite unlikely based on the finding that TDP-43 prefers long clusters of UG dinucleotide-rich regions [81, 82]. Further studies are necessary to understand the role of TDP-43 pathology in FTD/ALS and address the molecular mechanism stemming from the hexanucleotide repeats in c9FTD/ALS.

6. Therapeutic approaches

Current symptomatic treatments for unstable repeat expansion disorders provide only temporary and modest improvement in clinical symptoms without altering the natural course or outcome of the diseases. Although disease-modifying treatments are expected to slow early pathologic changes and disease progression, no cure or disease-modifying treatment is currently available. Therapy is focused on supportive care in order to relieve symptoms and improve quality of life. In this section, we will briefly review drugs and molecules being investigated in pursuit of disease-modifying strategies.

Many cognitive and behavioral aspects of FRAXA are thought to be attributable to excessive mGluR5 signaling system and can be reversed by down-regulation of mGluR5 [83]. It has been suggested that mGluR5 antagonists would be an attractive treatment for FRAXA [83, 84]. Fenobam, a highly selective mGluR5 antagonist [85], is under development for the treatment of FRAXA. A pilot single dose trial of fenobam did not show clinically significant adverse effects, which supports further clinical studies [86]. Lithium is an alternative drug which is currently available in humans. Lithium attenuates mGluR5 by down-regulating phospholipase C signaling

pathway [87]. Trial of lithium with young male patients with FRAXA was found to have significant improvement in verbal memory and behavioral functions [88].

Given that HD is caused by the mutation in a single gene, gene silencing can be an attractive therapeutic strategy [89]. Efforts have been made to accomplish this using antisense oligonucleotides or siRNAs [90, 91]. RNA interference directed against the mutant allele reduced disease progression and improved behavioral abnormalities in a transgenic mouse [92]. However, gene silencing of both mutant and normal alleles raises concerns about toxicity resulting from loss of normal protein functions. Furthermore, the major obstacle to gene silencing approaches is the requirement for adequate delivery of molecules into the disease cells [93]. Other approaches are aimed at improving cells' ability to survive in the presence of mutant Htt. These include correction of transcriptional regulation through histone deacetylase (HDAC) inhibition and modulation of HTT aggregation [94].

For RNA-mediated disorders, the elimination of the toxic mRNA would be a promising therapeutic target. In animal models of DM1, antisense technology demonstrated its proof of principle through various studies. A morpholino synthetic antisense oligonucleotide, CAG25, binds expanded CUG RNA and blocks its interaction with MBNL1. CAG25 dispersed nuclear RNA foci, released MBNL1 from sequestration, corrected the dysregulation of alternative splicing, and reversed ion channel function [95]. A modified antisense oligonucleotide also reduced significantly the level of toxic RNA and normalization of aberrant splicing without toxicity, demonstrating potential therapeutic use in other unstable repeat disorders [96]. However, clinical myotonia persisted in spite of normalized splicing of pre-mRNA. Further studies are needed to see if whether this approach could reverse cognitive symptoms.

Another therapeutic strategy is to identify small molecules which can directly interfere with the interactions of expanded nucleotide repeats and corresponding RBPs. Researchers from different groups have screened various compounds to identify molecules having inhibitory effects on the interactions between expanded repeats and MBNL1 [97-100].

CONCLUSIONS

The general underlying pathogenic mechanisms in unstable repeat expansion disorders include loss of protein function, gain of protein function, or altered function due to aberrant RNA-protein interactions. In diseases caused by a loss of function mechanism, progress has been made in understanding how loss of normal protein activity accounts for the phenotypes observed. In polyglutamine disorders caused by a gain of toxic function mechanism, significant emphasis has been placed on the expanded polyglutamine tract as the toxic moiety. The role of protein misfolding, altered function and accumulation has emerged as a point of overlap between polyglutamine disorders and RNA-mediated disorders. Whether it affects protein or RNA, this convergence indicates that repeat expansions might elicit a common cellular response. In polyglutamine disorders, altered conformation of the mutant protein triggers aberrant interactions leading to aggregation, whereas in RNA-mediated disorders, the proteins binding the mutant RNA might undergo a conformational change that interferes with their function, interactions, and clearance. It might be possible that expansions at the protein level could also have their RNA counterparts. However, it has also been suggested that multiple pathogenic mechanisms could coexist in a single disease [101, 102]. Further research should increase our understanding of protein and RNA interactions.

Intervention at an earlier stage like preclinical or prodromal stages in which a window of opportunity is open, prior to the perpetuation of a harmful cascade leading to neuronal dysfunction, may be critical. In this context, it is noteworthy that neurodegenerative dementias genetically caused by nucleotide repeat expansions have late onset of symptoms and delayed cell loss, which provides a significant window of opportunity for therapeutic intervention. Therefore therapeutic intervention at an earlier stage may significantly increase the likelihood that treatments will be effective to some extent and alter long term outcomes.

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