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RNA toxicity and foci formation in microsatellite expansion diseases

Nan Zhang[1,2] and **Tetsuo Ashizawa**[1,*]

[1]Neurosciences Research Program, Methodist Hospital Research Institute, Houston, Taxes, TX77030, US

[2]Division of Cell and Molecular Biology, South Kensington Campus, Imperial College London, London, SW7 2AZ, UK

Abstract

More than 30 incurable neurological and neuromuscular diseases are caused by simple microsatellite expansions consisted of 3–6 nucleotides. These repeats can occur in non-coding regions and often result in a dominantly inherited disease phenotype that is characteristic of a toxic RNA gain-of-function. The expanded RNA adopts unusual secondary structures, sequesters various RNA binding proteins to form insoluble nuclear foci, and causes cellular defects at a multisystem level. Nuclear foci are dynamic in size, shape and colocalization of RNA binding proteins in different expansion diseases and tissue types. This review sets to provide new insights into the disease mechanisms of RNA toxicity and foci modulation, in light of recent advancement on bi-directional transcription, antisense RNA, repeat-associated non-ATG translation and beyond.

Overview of microsatellite expansion diseases

Simple microsatellite expansions that cause neurodegenerative diseases can occur in coding regions [1–24], 5′-untranslated regions (5′-UTRs, [25–32]), intronic regions [33–51] and 3′-UTRs [24,50,52–62] (Table 1 and Fig. 1). In affected individuals, large repeat expansions show somatic and intergenerational instabilities and lead to disease phenotype. Several factors can contribute to repeat instability: sequence composition, convergent transcription, gene conversion, sister chromatid exchange, and errors in DNA replication, repair and mitotic recombination [63–66].

Repeat expansion in non-coding (and, perhaps, coding) regions often results in a toxic RNA gain-of-function (Table 1 and Fig. 1). In these disorders, the expanded RNA aggregates to form nuclear ribonucleoprotein foci which sequester RNA binding proteins (RBPs) or other essential cellular factors [67,68]. The depletion of key splicing regulatory RBPs from the cellular pool can lead to spliceopathy. Evidence to support such notion has been observed in Myotonic dystrophy type 1 (DM1), Spinocerebellar ataxia type 8 or 10 (SCA8/10), C9orf72-

*Corresponding author: Tetsuo Ashizawa, Neurosciences Research Program, Methodist Hospital Research Institute, Houston, Taxes, TX77030, US, Tel: 713-441-8224. tashizawa@houstonmethodist.org.

Conflict of Interest

The authors declare no conflict of interest.

amyotrophic lateral sclerosis/frontotemporal dementia (C9orf72-ALS/FTD) and Fuchs endothelial corneal dystrophy (FECD) [37,51,69–71]. RBPs also participate in cellular processes such as DNA repair, transcription regulation, RNA processing/transport/localization, microRNA (miRNA) processing, protein quality control and apoptosis [72–74]. Disruption of such processes by foci sequestration may further exacerbate neuronal toxicity. As bidirectional translation occurs at most non-coding expansion regions, mechanisms such as antisense foci formation, RNA-induced silencing complex (RISC)-dependent RNA degradation, siRNA-dependent epigenetic modification and repeat-associated non-ATG (RAN) translation may also occur [34,75–78]. Theoretically, nuclear RNA foci could provide neuroprotection by acting as a toxic RNA sink, so that the generation of RAN-translated toxic polypeptides within the cytoplasm is reduced [79]. RNA Foci may also function as a 'triage' site – reminiscent to the role of stress granules – to determine the destination of the expanded RNA. In this review, we will focus on RNA toxicity and foci formation.

RNA toxicity and foci in DM

DM1 and DM2 (Myotonic dystrophy type 2) are multisystem diseases that target tissues including skeletal, cardiac and smooth muscles, the central nervous system (CNS) and eyes. DM2 has a milder clinical course than DM1. The genetic origin of DM stems from a CTG repeat expansion in the 3′-UTR of the *DMPK* gene for DM1 and a CCTG expansion in intron 1 of the *CNBP/ZNF9* gene for DM2.

A hallmark of DM pathology is the formation of toxic nuclear RNA foci. In DM1, the CUG-expanded RNA forms hairpins that sequester a class of splicing regulatory RBPs – Muscleblind 1-3 (MBNL1-3) – at the periphery of the nuclear splicing speckles [80,81]. MBNL1 participates in foci formation by binding to distorted GC bases or unpaired UU bases [82–85]. Its depletion has recently been linked to mRNA mislocalization and miRNA misprocessing [86,87]. In DM1, stabilization of another splicing regulator CUG-binding protein 1 (CUGBP1 or CELF1) also occurs [88]. Changes of MBNL1 and CUGBP1/CELF1 in DM1 drive splicing of a variety of transcripts towards fetal isoforms. Many of the misspliced genes are components of the sodium/calcium current regulation, intra-/inter-cellular transport, and sarcomere/cytoskeleton structure and function [89,90]. They are thus unable to fulfill their normal cellular functions.

Recently, antisense CAG-expanded foci have been reported in adult and congenital DM1 patients and mice [91,92]. These foci do not appear to colocalize with sense foci in the same nucleus nor sequester any MBNL (Fig. 2). As antisense foci exist at much lower numbers than sense foci, therapies using antisense oligonucleotides that target sense foci may change the relative amount of sense *vs.* antisense foci. In DM2, CCUG foci appear much larger than DM1 foci and contain only intronic repeats. Several foci modifiers exist in DM. For instance, two DM1 RNA toxic foci enhancers (*str-67* and *ocrl-1*) have been identified in *Caenorhabditis elegans* [93]. Nonsense-mediated decay, Staufen and DEAD-box helicase 5 (DDX5) all have an impact on toxic RNA transport and degradation [94–96]. In addition, a large ribonucleoprotein complex may transiently regulate the sense foci (Fig. 2, [97]).

DM foci are incredibly dynamic during cell proliferation [39,62]. Hence, caution should be taken when foci counts are used as a biomarker in non-synchronized cells. DM foci are most prominent at early prophase. The majority of foci disappear during nuclear membrane breakdown. When cells exit mitotic division, foci progressively accumulate in the nucleus.

RNA toxicity and foci in C9orf72-ALS/FTD

ALS and FTD are neurodegenerative diseases that share clinical and pathological overlap. A subset of these diseases have been linked to a common causal GGGGCC repeat expansion in intron 1 of *C9orf72*. An RNA toxic role has been implicated in C9orf72-ALS/FTD, because the expanded RNA binds to a variety of RBPs [78], and the expanded sense RNA alone is sufficient to recapitulate neurodegeneration in *Drosophila* and BAC mice [98,99]. Both sense and antisense nuclear foci have been found in patient tissues, induced pluripotent stem cells (iPS cells) and iPS-derived neurons, *Drosophila* and BAC mice [24,35,98,100–103]. Sense foci contain GGGGCC repeat sequences [100], and can induce apoptosis in SH-SY5Y cells and zebra fish embryos [104]. Antisense foci preferentially accumulate in vulnerable cell types [98], and correlate with a nuclear loss of TAR DNA-binding protein 43 (TDP-43) in motor neurons [35]. The above observations suggest that antisense foci play a significant role in C9orf72 pathophysiology. In contrast to DM1 foci, both sense and antisense *C9orf72* foci can colocalize within the same nucleus [105].

Various interactomes containing *C9orf72* RNA and RBPs have been proposed. It has been confirmed that both sense and antisense foci or RNA bind to Serine and Arginine-rich splicing factor 2 (SRSF2), AlY/REF export factor (ALYREF), Heterogeneous nuclear ribonucleoprotein F (hnRNP F) and hnRNP A1 [35,106]. It is unclear as to whether RNA secondary structure is a determinant in RBP recognition *in vivo*, because only the sense *C9orf72* RNA has been verified to form G-quadruplexes *in vitro* [48], and one cannot rule out a shared secondary structure between sense and antisense C9orf72 RNA foci *in vivo*. Sense foci also colocalize with Purine-rich element binding protein-alpha (Purα), Ran GTPase activating protein 1 (RanGAP1) and Adenosine deaminase B2 (ADARB2) [100,107,108]. Purα may recognize partially denatured RNA in a similar way as MBNL1 [83,109]. ADARB2 may participate in foci formation or maintenance, and its sequestration leads to hypersensitivity to excitotoxicity [100]. Recently, a comparative analysis has been done on RBPs that bind to *C9orf72* RNA in five studies [78]. hnRNP H shows most overlap across studies, followed by Splicing factor proline and glutamine-rich (SFPQ), Interleukin enhancer binding factor 2 (ILF2) and Myelin basic protein (MBP). Interestingly, SFPQ regulates *ADARB2* expression and is essential for paraspeckle formation [110].

RNA toxicity and foci in Fragile X-associated tremor/ataxia syndrome (FXTAS)

FXTAS is an adult-onset neurodegenerative disease with clinical manifestations of tremor, gait ataxia, parkinsonism and cognitive impairment caused by a CGG intermediate expansion at the 5′-UTR of *FMR1*.

Several lines of evidence point to an RNA gain-of-function in FXTAS. First, expression of the expanded CGG irrespective of its genetic context is sufficient to cause neurodegeneration in mice and *Drosophila* [111,112]. Second, transcription efficiency of *FMR1* is significantly elevated in FXTAS patients and mice, while the FMR protein (FMRP) level remains largely unchanged [113–115]. Third, co-expression of both CCG and CGG-containing RNA suppresses their independent toxicity [116]. Fourth, large ubiquitin-positive nuclear inclusions have been found in FXTAS tissues. These nuclear inclusions contain CGG expanded transcripts in post-mortem FXTAS brains but not in *Drosophila* [117–119]. However, the number of inclusions correlates positively with CGG repeat length in *Drosophila* [120].

Nuclear CGG foci/inclusions contain a number of constituents in FXTAS *Drosophila*, mice and patients (Fig. 2, [28,117,121–123]). Amongst these RBPs, overexpression of DGCR8, Pura or hnRNP A2/B1 has been linked to suppression of neurodegeneration or RNA toxicity [28,122,123]. Additionally, a 'sequential recruitment' model has been proposed via DGCR8/DROSHA and Sam68 (Fig. 2). However, the pathogenic role of DGCR8/DROSHA remains controversial [124–127]. It has recently been shown that TDP-43 suppresses the CGG repeat-induced RNA toxicity through hnRNP A2/B1 but not through direct RNA interactions [128].

RNA toxicity and foci in SCA8

SCA8 is caused by a CTG expansion in the 3′-UTR of *ATXN8OS* (Table 1). Clinical manifestations of SCA8 include gait and limb ataxia and cerebellar atrophy on MRI imaging. In contrast with most other non-coding expansion diseases, little evidence has linked repeat length to disease severity and progression in SCA8 [129].

RNA gain-of-function plays a significant role in SCA8. It has been shown that a $(CUG)_{400–1000}$ expansion forms nuclear RNA foci in human SCA8 brains. These foci vary greatly in size, distribution and number: While multiple small CUG foci were found in Purkinje cells, single CUG foci were found in the nuclei of molecular layer interneurons and the Bergmann glia in the granule cell layer [70]. In both human and mice, MBNL1 colocalizes with CUG foci in molecular layer interneurons but not in Purkinje cells. The pathogenic significance of such tissue-specific sequestration pattern is unknown. Mutations in MBNL1 and other expansion modifiers have been shown to enhance SCA8-induced neurodegeneration synergistically in *Drosophila* [130]. CUG expansion also increases the expression of CUGBP1/MBNL1-regulated *Gabt4*, leading to a loss of GABAergic inhibition [70].

RNA toxicity and foci in SCA10

SCA10 is prevalent in Latin America and primarily impairs cerebellar Purkinje cells [131,132]. The disease-causing mutation is an ATTCT repeat expansion in intron 9 of *ATXN10*, which is likely to have originated in the Han Chinese population [133]. The expanded AUUCU repeats form RNA hairpins with UCU internal loops closed by AU pairs [134]. In a subset of SCA10 patients, the presence of interruption motifs within repeat

expansions correlates strongly with epileptic seizures [135–137]. A similar correlation between repeat interruption and disease phenotype has been observed in DM1 [138]. In contrast, the presence of interruptions reduces pathogenicity in CAG-expanded SCA 1/17 and penetrance in SCA8 [139].

The spliced AUUCU repeat expansion is the principle pathogenic molecule that triggers neuronal death in SCA10 [51]. Both $(AUUCU)_{1000–2000}$ and ectopically expressed $(AUUCU)_{500}$ form nuclear and cytoplasmic foci in human cells and transgenic mouse brains. Nuclear AUUCU foci have been shown to colocalize with hnRNP K (Fig. 2). Sequestration of hnRNP K not only alters splicing regulation, but also releases Protein kinase C-delta (PKCδ) [51]. Translocation of PKCδ to mitochondria causes cytochrome c release and activation of caspase-3, leading to apoptosis [140].

RNA toxicity and foci in SCA31 and SCA36

SCA31 is caused by a TGGAA repeat expansion in the bidirectionally transcribed *BEAN* gene. The reverse complementary sequence of the SCA31 repeat ($TTCCA.TTCCA_n$) matches a tandem interruption motif at the 3′-end of *SCA10* subtype B [136]. The GGCCTG expansion in SCA36 matches a hexanucleotide interruption motif as seen in DM1 and differs by 1 base from the *C9orf72* GGGGCC repeat (SCA36: $GGCCTG.GGCCTG_n$) [141]. Given the above information, it is possible that the pathogenic pathways and target RBPs are shared between SCA10 and SCA31, and between C9orf72-ALS/FTD and SCA36.

Conclusions and future perspectives

In conclusion, RBPs play key roles in toxic RNA gain-of-function in non-coding expansion diseases. However, several factors warrant further investigation. (1) RAN translation has been implicated in non-coding expansion disorders including DM1, SCA8, C9orf72-ALSFTD and FXTAS (Table 1, [77]). (2) RNA foci bear extensive structural similarities to RNA transport granules, stress granules, nuclear bodies, and P bodies, as these structures all contain a large population of localized mRNA and associated RBPs [142,143]. Can RNA toxicity be extended to RNA transport, sorting and degradation pathways? (3) RBPs (such as MBNL, Staufen and FMRP) and alternative last exons near 3′-UTRs are important for guiding mRNA to neurites [87,143,144]. Disruption of mRNA localization patterns by RBP sequestration and 3′-UTR repeat expansion may contribute to disease phenotype [145]. (4) Several mechanisms may actively facilitate RNA nuclear retention. For instance, hnRNP H inhibits nuclear export of the expanded RNA in DM1 [146]. Bulged stem-loop structures that resemble repeat expansions at 5′- or 3′-UTRs have been predicted to guide mRNA nuclear localization [125]. (5) G-rich repeat expansions, such as GGGGCC, may be taken up by lysosomes via LAMP2C receptors [147], leading to over-capacitated autophagy and non-degraded toxic products. (6) Deleterious R-loops may form at CTG, CCG, CAG, CGG, GAA and GGGGCC repeat sites. When not resolved by RNase H, R-loops can affect chromosome stability, Ig class switching, mitochondrial DNA replication and telomeric transcription [148,149]. (7) The expanded antisense RNA may facilitate sense foci degradation via nuclear and cytoplasmic RISC complexes [150]. It may alternatively form antisense foci that correlate with disease severity [35,98]. The abovementioned factors may

serve as additional drug targets, depending on their relative contribution to the overall pathologic mechanisms of microsatellite expansion diseases. A comprehensive analysis of these factors will also help us better understand the complexity and development of microsatellite expansion diseases and identify the linkage between toxic RNA and disease etiology at a molecular level.

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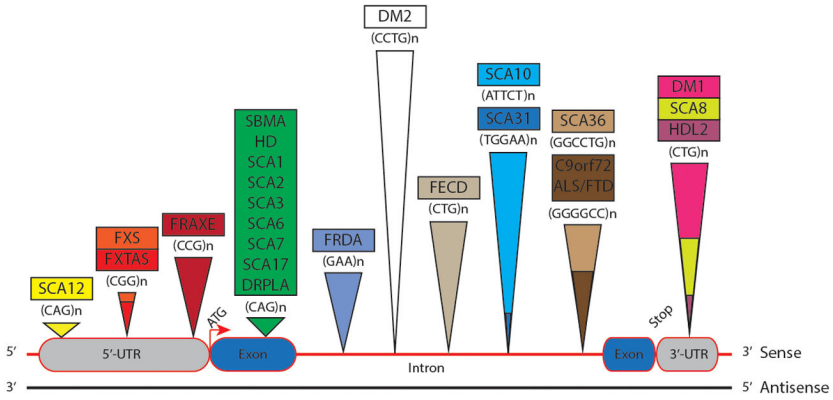

Fig. 1.

Schematic illustration of repeat expansion diseases. Repeats associated with an RNA gain-of-function are located in: (1) the 5′-UTRs, such as spinocerebellar ataxia (SCA) type 12 and Fragile X-associated tremor/ataxia syndrome (FXTAS), (2) intronic regions, such as myotonic dystrophy type 2 (DM2), Fuchs endothelial corneal dystrophy (FECD), SCA10/31/36 and C9orf72-amyotrophic lateral sclerosis/frontotemporal dementia (C9orf72-ALS/FTD), and (3) the 3′-UTRs, such as myotonic dystrophy type 1 (DM1), SCA8 and Huntington disease-like 2 (HDL2). In HDL2, the CTG repeat occurs in an alternatively spliced exon 2A, generating either a 3′-UTR CTG expansion (as shown in this figure) or homopolymers. Fragile X mental retardation syndrome (FXS), Fragile XE syndrome (FRAXE) and Friedreich ataxia (FRDA) are exceptions where an RNA loss-of-function occurs due to transcription silencing by CGG, CCG and GAA expansions respectively. The polyGlutamine (polyQ)-coding CAG expansions occur in exons and may involve RNA-mediated toxicity. These disorders include spinal and bulbar muscular atrophy (SBMA), Huntington's disease (HD), SCA1/2/3/6/7/17 and dentatorubral-pallidoluysian atrophy (DRPLA). CAG repeat RNA-based toxicity has been suggested in some of these disorders [151,152]. The repeat length in affected individuals correlates approximately with the height of the triangle.

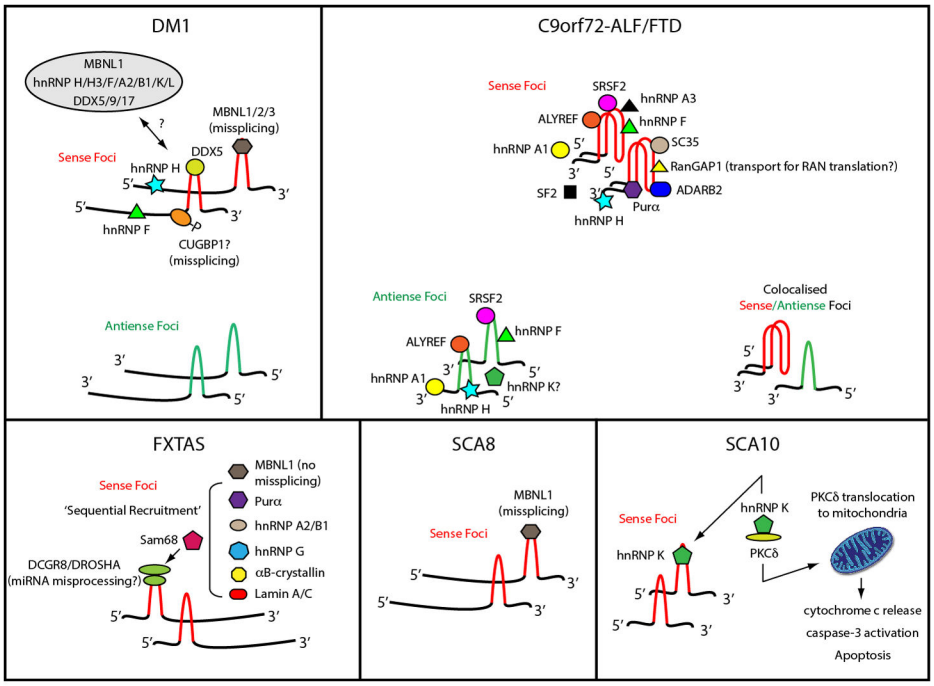

Fig. 2.

Schematic illustration of RNA foci and associated RBPs in DM1, C9orf72-ALF/FTD, FXTAS, SCA8 and SCA10. This diagram only included RBPs that had been verified by *in vivo* colocalization experiments. Repeat expansions present in sense and antisense RNA are highlighted in red and green respectively. Hyperphosphorylated (P) CUGBP1 may bind to the base of *DMPK* hairpins in DM1. The expanded *C9orf72* sense RNA assumes an unusual G-quadruplex structure. In FXTAS, the miRNA processing complex DGCR8/DROSHA is among the first proteins to be recruited [28]. The DGCR8/DROSHA heterodimer is partially sequestered because it can still process miRNA to some extent. Sam68 binds to DGCR8/DROSHA and recruits other RBPs [29,117].

Table 1

Summary of microsatellite expansion disease mechanisms. The following abbreviations are indicated: AS – antisense, t – *in vitro*, v – *in vivo*, D – downregulation, and U – upregulation. References are indicated in the disease overview section of the manuscript.

Disease	Repeat expansion (healthy/affected)	Location of expansion	Antisense transcript	RNA foci	RBPs bound to expanded RNA or foci	RAN translation	miRNA dysregulation
C9orf72-ALS/FTD	GGGGCC (2–19/250–1600)	Intron 1 of *C9orf72* (9p21.2)	*C9orf72AS*	Sense & antisense foci in both nucleus & cytoplasm	RanGAP1, hnRNP A1/F/H/K?/U, SRSF2, ALYREF, Purα, nucleolin, RPL7^{t}; RanGAP1, SF2, SC35, hnRRNP A1/A3/F/H/K?, ADARB2, Purα, SRSF2, ALYREF^{v}	PolyGA/GP/GR/PR/PA^{t}; PolyGA/GP/GR/PR/PA^{v}	?
DM1	CTG (5–37/50->4000)	3′-UTR of *DMPK* (19q13.32)	*DMPKAS*	Sense & antisense foci in both nucleus & cytoplasm; non-toxic cytoplasmic foci	MBNL1/2/3, CUGBP1, DDX5/6/17^{t}; MBNL1/2/3, DDX5, hnRNP H/F^{v}	PolyQ/A/S^{t}; PolyQ^{v}	miR-29b/29c/33/100/22/27a/let-7d/let-7b/194/148a/30b/125b/23a/499/365/23b/328/26a/let–7 g/145/133a/1^{D}; miR-1/335/9/135a/206^{U}; mislocalization of myomiR-1/133b/206
DM2	CCTG (<30/75–11,000)	Intron 1 of *CNBP/ZNF9* (3q21.3)	?	Sense foci in both nucleus& cytoplasm	MBNL1/2/3, CUBGP1, 20S catalytic core of proteasome, eIF2^{t}; MBNL1/2/3^{v}	?	miR-125b-5p/193a-3p/193b-3p/378a-3p^{D}; miR-34a-5p/34b-3p/34c-5p/146b-5p/208a/221-3p/381^{U}
DRPLA	CAG (6–35/49–88)	Exon 5 of *ATN1* (12p13.31)	?	?	?	?	Dysregulation of miR-200b/429?
FECD	CTG (<40/50–2600)	Intron 3 of *TCF4* (18q21.2)	?	Nuclear sense foci	MBNL1^{v}	?	miR-199b-5p/616-3p/29a-3p/140-3p/184/30c-5p/30b-5p/101-3p/502-5p/181a-5p/455-5p/340-5p/130b-3p/95/484/532-3p/15b-5p/195-5p/34a-3p/628-5p/330-3p/374b-5p/502-3p/148b-3p/185-5p/107/191-5p/1244/374a-5p/135a-5p/186-5p/125b-5p/181c-5p/660-5p/125a-5p/30d-5p/7-1-3p/378a-5p/29b-2-5p/671-3p/26a-5p/7d-5p/30a-5p/26b-5p/340-3p/93-3p/148a-3p/151a-3p/151a-5p/99a-5p/7g-5p/532-5p/23b-3p/19a-3p/362-5p/30e-3p/361-5p/149-5p/100-5p/99b-5p/483-3p/574-3p/455-3p^{D}
FRAXE	CCG (10–35/300–1200)	5′-UTR of *AFF2/FMR2* (Xq28)	*FMR3*	?	?	?	?

Disease	Repeat expansion (healthy/affected)	Location of expansion	Antisense transcript	RNA foci	RBPs bound to expanded RNA or foci	RAN translation	miRNA dysregulation
FRDA	GAA (5–30/70->1000)	Intron 1 of *FXN* (9q21.11)	*FAST-1*	No detection of foci	?	?	miR-500/614/944/132/9/125b/335/126/323/9[D]; miR-886-3p/886-5p/923/518f/363/21/124/130a/10a/505/643/155/628-3p/194/20b/192/181a[U]
FXS	CGG (5–44/>200)	5′-UTR of *FMR1* (Xq27.3)	*ASFMR1, FMR4 & FMR6*	?	?	?	miR-382/134/124/370/431/494/372/373[D]; miR-125b/138/128[U]
FXTAS	CGG (5–44/55–200)	5′-UTR of *FMR1* (Xq27.3)	*ASFMR1, FMR4 & FMR6*	Nuclear sense foci	MBNL1, SRSF1/4/5/6/7/10, nucleophosmin, tropomycin, actin, keratine, tubulin, MAP6, SH3BP5, HBB, STRBP, NF-κB, DDX3/5/17, ILF2, MTA2, Hsp27, GMDS, ubiquitin, hnRNP A1/A2/B1/A3/C/D/E1/G/M, Purα, ESRP1, PRP3, ZC3HA, LSM11, ZCHC8, MPP10, DAZP1, RBMS1, GLD2, DGCR8[t]; MBNL1, hnRNP A2/B1/G, Purα, DGCR8, lamin A/C, aB-crystallin, Sam68[v]	PolyG/PolyA[t]; PolyG[v]	miR-221/455-5p/206/502-3p/193a-5p/449a/378/486-3p/3065-5p/135b/30b/550a/337-3p/502-5p/654-5p/let-7i[D]; miRNA-935/1238/133a/582-5p[U]
HD	CAG (6–37/35–121)	Exon 1 of *HTT* (4p16.3)	*HTTAS*	Nuclear sense foci; no detection of antisense foci	HTT, MBNL1[v]	PolyA/S/L/C[t]; PolyA/S/L/C[v]	miR-22/29c/128/132/138/218/222/344/674/9/125b/138/146a/150/218/221/222/100/135a/135b/181c[D]; miR-1273p/145/148a/199-5p/199-3p/200a/205/214/335/34b[U]
HDL2	CTG (6–28/>41)	3′-UTR or exon 2A of *JPH3* (16q24.2)	*JPH3AS*	Nuclear sense foci containing unprocessed JPH3 RNA	MBNL1[v]	PolyQ/A/S[t]; PolyQ[v]	?
SBMA	CAG (<36/38–62)	Exon 1 of *AR* (Xq12)	?	Nuclear sense foci	?	?	Dysregulated miRNA-298
SCA1	CAG (6–44/>39)	Exon 8 of *ATXN1*	*ATXN1AS*	?	?	?	miR-19/101/130[D]
SCA2	CAG (13–31/32–79)	Exon 1 of *ATXN2* (12q24.12)	?	?	?	?	miR-12/bantam/sickle[U]

Disease	Repeat expansion (healthy/affected)	Location of expansion	Antisense transcript	RNA foci	RBPs bound to expanded RNA or foci	RAN translation	miRNA dysregulation
SCA3	CAG (12–44/60–87)	Exon 8 of *ATXN3* (14q32.12)	?	Nuclear sense foci; no detection of antisense foci	MBNL1[v]	PolyQ/A/S[t]	miR-125b/bantam/29a/let-7f/215/342-3p/429/193a-5p/98/223/375/500a/Plus-E1247/19b/Plus-F1074/374a/192/324-3p/19a/205/200a/18a/142-5p/125b/18a/27a/1297/25[D]; miR-34b/647/H8/620/Plus-E1097/576-5p/Plus-E1146/H6-3p/138-1/1260/1258/431/1261/574-3p/659/1264/Plus-E1110/888/935/625[U]
SCA6	CAG (<18/20–33)	Exon 47 of *CACNA1A* (19p13.13)	?	?	?	?	?
SCA7	CAG (<19/>36)	Exon 3 of *ATXN7* (3p14.1)	*SCAANT1*	?	?	?	?
SCA8	CTG (15–50/71–1300)	3′-UTR of *ATXN8OS* (13q21.33)	*ATXN8 & KLHL1*	Nuclear sense foci	MBNL1[v]	PolyQ/A/S/L/C[t]; PolyA[v]	?
SCA10	ATTCT (10–31/800–4500)	Intron 9 of *ATXN10* (22q13.31)	?	Nuclear & cytoplasmic sense foci	hnRNP K[tv]	?	?
SCA12	CAG (7–32/46–78)	5′-UTR of *PPP2R2B* (5q32)	?	?	?	?	?
SCA17	CAG (25–44/47–63)	Exon 3 of *TBP* (6q27)	*PCD2*	?	?	?	miRNA-29a/b[D]; miRNA-322[U]
SCA31	TGGAA (0/>110)	Intron shared by *BEAN & TK2* (16q21)	*TK2*	Nuclear sense foci; no detection of antisense foci	SRSF1/9[t]	?	?
SCA36	GGCCTG (5–14/650–2500)	Intron 1 of *NOP56* (20p13)	?	Nuclear & cytoplasmic sense foci	SRSF2[tv]	?	miRNA-1292[D]