Article title: The quest towards understanding the molecular pathogenesis of triplet repeat disorders: Huntington's Disease and Fragile X-Associated Tremor and Ataxia Syndrome

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The quest towards understanding the molecular pathogenesis of triplet repeat disorders: Huntington’s Disease and Fragile X-Associated Tremor and Ataxia Syndrome

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Abstract

Trinucleotide repeat disorders encompass a group of neurological diseases driven by unstable repeat expansions. Huntington’s disease (HD) is characterized by chorea and brain atrophy. The normal huntingtin protein contains 6-34 CAG repeats; however, upon a threshold effect that exceeds 36 repeats, HD becomes fully penetrant and promotes degeneration of neuronal populations. In contrast, FXTAS arises from polyglycine repeats between 22-200. FXTAS carriers experience severe tremor, ataxia and brain atrophy. Here, we analyze the various ways mutant huntingtin and FMR1 mRNA aggregates induce intracellular dysfunction in HD and FXTAS, specifically in the context of impaired neuronal processes and the protein-protein interactions. The identification of these molecular processes offers potential targets for drug intervention and could lead to the development of innovative therapies to treat HD and FXTAS neuropathogenesis. (200)
Trinucleotide repeat disorders

Introduction

Trinucleotide repeat disorders (TRDs) encompass neurological inherited diseases mediated by unstable repeat expansions (Figure 1). TRDs are classified into coding and noncoding disorders (1). Coding disorders include polyglutamine expansion, such as Huntington’s Disease (HD). Noncoding disorders include polyglycine expansions, such as Fragile-X-Associated Tremor and Ataxia Syndrome (FXTAS).

HD is an autosomal dominant disorder induced by CAG expansion located within the open-reading frame of exon 1 of the HTT gene (2). The normal huntingtin gene contains 6-34 CAG repeats; however, upon a threshold effect that exceeds 36 repeats, HD becomes fully penetrant and promotes neuronal degeneration, this induces chorea and progressive cognitive deficits (2). Although the function of wild-type (WT) huntingtin (Htt) is unknown, huntingtin is expressed within the brain and interacts with structures such as the mitochondria and microtubules (3). There is no treatment for HD and death occurs within 10-15 years. HD age-of-onset inversely correlates with CAG length; however, individuals with longer repeats can experience earlier age-of-onset (2). CAG repeats >28 experience greater instability upon replication which promotes polyglutamine expansion. This accounts for anticipation (4), in which age-of-onset occurs earlier in successive generations.

FXTAS is caused by a “premutation” CGG expansion located within the 5’ untranslated region of the FMR1 gene (5). The normal FMR1 gene contains between 5-45 CGG repeats; however, when the polyglycine expansion is between 55-200, it becomes pathogenic (5). In contrast, CGG expansions >200 result in full gene mutation and lead to Fragile-X-Syndrome. FXTAS is characterized by tremor, gait ataxia, cognitive deficits and brain atrophy (5). As with HD, FXTAS age-of-onset inversely correlates with increased CGG repeats. Furthermore, CGG repeats express anticipation (6). Although the WT function of FMR1 gene protein (FMRP) is unknown, it is thought to be involved in synapse development and translation (5).

A neuropathological hallmark of HD and FXTAS includes the formation of protein and mRNA inclusion bodies (IBs), respectively, within the brain (2,5). At the molecular level, CAG
expansions and \textit{FMR1} CGG premutation carriers express a 2-8-fold increase in huntingtin and \textit{FMR1}mRNA, respectively (7,8). Although the role of IBs in HD and FXTAS is unknown, various proteins and RNAs have been found sequestered into the IBs. In this review, the mechanism of aggregate formation is briefly reviewed. The ways mutant huntingtin (mHtt) and \textit{FMR1}mRNA aggregates induce intracellular dysfunction in HD and FXTAS is analyzed, specifically in the context of impaired neuronal processes and the protein-protein interactions within the proteostasis network. The identification of these molecular processes offers targets for drug intervention and therapy development to treat HD and FXTAS.

Figure 1. Trinucleotide repeat expansion disorders are caused triplet repeat expansions in coding and noncoding gene region. Examples of some known TRDs, repeat expansion threshold and associated symptoms are shown. Huntington’s disease is a coding TRD encoding an expanded polyglutamine tract. Fragile-X Associated Tremor and Ataxia Syndrome is a non-coding TRD encoding an expanded polyglycine tract. Currently, gain-of-function model is a contender for cell toxicity; however, RAN-based translation peptides has been shown to be implicated in FXTAS pathogenesis and possibly in polyglutamine TRDs. TRDs can also arise from sense-antisense transcription; however, in this diagram only the sense strand is shown. TRDs have been shown to have a significant impact on human health and are responsible for many neurological diseases. Inheritance these genes can also cause rapid gene expansions; resulting in anticipation. DM, myotonic dystrophy; FRDA, Friedreich ataxia; FXS, fragile X syndrome; FXTAS, fragile X associated tremor and ataxia; OPMD, oculopharyngeal muscular atrophy; SCA, spinocerebellar ataxia; TRD, triplet repeat disorders; UTR, untranslated region. (2,5, 68)

Methods

PubMed and Google Scholar databases were used to retrieve articles using keywords relevant to the molecular pathogenesis and neuropathology of HD and FXTAS (e.g.,
“Huntington’s Disease” AND/OR “molecular pathology” AND/OR “neuropathology” AND/OR “therapeutics” the same was applied for FXTAS). Inclusion criteria: peer-review and primary literature. Database filters were applied to include articles released after the 1990s due to the pioneering work performed on these diseases.

**Mechanisms of Aggregate Formation**

**The polar zipper and gain-of-function model**

The polar zipper model states that huntingtin conformation is destabilized by expanded polyglutamine tracts, which lead to abnormal mHtt-mHtt interactions (2) (Figure 2). Polyglutamine protein-protein interactions induce the formation of insoluble β-pleated sheets via hydrogen bonding (2). McGowan et al. (9) stained IBs of HD brain samples with Congo Red, which resulted in dye polarization, a characteristic state of proteins with β-pleated sheets. Furthermore, a positive correlation was identified between increased repeat size and β-hydrogen bond formation (10). Although this model is an attractive theory, it fails to consider the interactions between CAGs and other proteins. This gain-of-function mechanism might explain some aspects of FXTAS-mediated toxicity, given multiple proteins have been identified to co-localize in mRNA aggregates (Figure 3).

**The transglutaminase model**

Transglutaminases (TGs) are enzymes that mediate the crosslinking of glutamine-glutamine residues via covalent ε-lysine residues (2) (Figure 2). Cortex HD brain sample analyses revealed that 99% of TG-2 activity co-localized with mHtt (11). Polyglutamine tracts might increase glutamine-transglutaminase crosslinking activity, resulting in IBs. The transglutaminase model is an appealing theory for aggregate formation; however, some data contradict this model. Huntingtin IBs might also result from TG-mediated polyamination (12). In support of this, 1mM of putrescine was seen to almost inhibit tau crosslinking and given polyamines are found within the millimolar range (12), thus; mHtt-polyamination cannot be ruled out.
Repeat associated non-ATG translation (RAN) model

The repeat associated non-ATG translation (RAN) model describes a non-ATG mediated 5’UTR FMR1 translation of toxic proteins (5) (Figure 3). RAN-based CGG translation occurs in two FMR1 transcript reading frames. FMRpolyG is the main RNA translation product, while FMRpolyA is the second (5). FXTAS brain sample analyses identified FMRpolyG/A-nuclear aggregates that cosegregate with ubiquitin- and heat-shock-positive inclusions (13,14). The role RAN-based translation products play in FXTAS pathogenesis is unknown; however, protein co-localization within aggregates suggests that these products interact with proteins whose normal function is impaired upon sequestration. The RAN translation model displays a different gain-of-function model, which might mediate FXTAS pathogenicity.
**Intracellular dysfunction in HD and FXTAS**

Polyglutamine and polyglycine expansion in HD and FXTAS, respectively, is hypothesized to result in a gain-of-function mechanism that is toxic to the cell. In contrast, loss of wild-type function disrupts homeostasis. The contribution of protein and mRNA toxicity to the deregulation of intracellular pathways is analyzed.

**Huntington’s disease, heat-shock proteins and transcription factors**

Huntingtin aggregation is polyglutamine length-dependant and co-localizes with heat-shock proteins (HSPs). HSPs are chaperone proteins that prevent protein misfolding and aggregation (2). Transfection of three different cell lines with GFP-mHtt of various polyQ lengths induced aggregate formation and HSP co-localization across all cell lines (15). Apart

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Figure 3. Schematic representation of RNA-gain of function model and RAN-based translation model. A. RNA-gain of function model displays how proteins can be sequestered by binding to polyglycine repeats directly or indirectly (e.g. Purα-Rm62). This depletes bound proteins from their protein pools, decreasing their interactions with target substrates and activating a downstream effect of impaired pathways. B. Repeat associated non-ATG translation (RAN) model. The polyglycine repeat is believed to induce the formation of a hairpin structure, as the translation initiation complex binds it stalls due to the hair-pin, promoting non-ATG based translation with similar start codons (CTG), this results in a frame-shift producing FMRpolyG, FMRpolyA or rarely FMRpolyR, these proteins can then sequester proteins in a similar model as RNA-gain of function seen in (A) and in HD. Figure adapted from (52).
from HSP-co-localization, analyses of mice neuroblastoma cells(CAG96) and yeast-models(CAG96) identified the co-localization of TATA box binding protein (TBP), a transcription factor (TF) required for protein transcription (15). Schaffar et al., (15) suggested that mHtt-TBF interaction is due to polyglutamine-polyglutamine interactions, resulting in Beta-sheet folding and aggregation. Indeed, live neuronal imaging identified the N’terminus TBP domain PolyQ tract to co-localize with mHtt aggregates (16), suggestive of transglutaminase-mediated aggregation. Furthermore, TBP-associated factor-4(TAF4) was seen to co-localize with mHtt aggregates (17). This is important given TBP-TAF4 comprises transcription factor II D (TFII D), a transcription complex required for PGC-1α transcription (17). PGC-1α is a TF that regulates metabolic processes. A PGC-1α mRNA analysis of presymptomatic HD striatum samples displayed a 30% activity decrease (17). These results indicate that PGC-1α expression is decreased in the caudate, one of the first regions known to degrade in HD (18). While TAF4 overexpression in mHtt(Q11) striatal cells reversed huntingtin-induced PGC-1α inhibition (17). These findings indicate that mHtt-mediated TBP-TAF4 sequestration impairs TFII D gene transcription, resulting in alterations of energy metabolism genes. Ultimately, energy and mitochondrial dysfunction increases reactive-oxygen species, leading to neuronal death.

Interestingly, a gene-set enrichment analysis of PGC-1α-KO mice revealed how closely downregulated pathways in the mice matched those from early-stage HD patients (19). These findings suggest that decreased PGC-1α might represent an early defective step in HD.

Previous studies have suggested that HSP-overexpression decreases huntingtin aggregate formation. For example, HSP40/70 overexpression decreased aggregation with no TBF co-localization (15), which suggests that HSP40/70 interferes with polyQ toxicity and protein-binding. Although HSP overexpression might decrease aggregation, there is contradictory data. For example, proteasome purification of insoluble fractions of an R6/2 HD mouse model identified 1.5% of the content to be HSP40, -70 and -90 (19). There are some possible explanations for the discrepancies between studies. First, different cell lines used in the studies might express different HSPs that alter IB formation (15,20). For example, antagonist interactions between HSP104 and HSP70 suggest that polyQ aggregation depends on their overall balance (20). While transfection of different vectors might impact different set
of genes. Although induced expression of heat-shock factor1 and heat-shock response increased the R6/2 HD mouse model lifespan, aggregate reduction was transient and disappeared during disease progression (21). These findings indicate that throughout disease progression, HSPs attempt to unfold mHtt but become sequestered into IBs, implying that their effects are compensatory.

**Huntington’s disease, heat-shock proteins and transcription factors**

Huntingtin polyglutamine expansions impair the heat-shock response (HSR). HSF1 is a TF involved in the activation of an HSR and HSPs (22). Genome-wide chromatin immunoprecipitation of mHtt(Q111) striatal neurons displayed decreased HSF1 activity capable of binding 39% of target genes (22). These results suggest that mHtt fails to activate an HSR. Recent studies have implicated a role for CK2α’kinase and Fbxw7 (23). CK2α’kinase and Fbxw7 are proteins that phosphorylate and stimulate ubiquitin-dependent degradation. Medium spiny neurons (MSNs) of HD mice models(Q175) and HD patients’ striatal tissues exhibited increased CK2α’ and Fbxw7 levels (23). Mass spectrometry analysis revealed that CK2α’ phosphorylates HSF1 at S303 and S307 sites, promoting its degradation. Furthermore, Fbxw7 removal also increased HSF1 levels. Gomez-Pastor et al., (23) identified a mechanism of defective HSF1 activation, given mHtt targets HSF1 for proteasomal degradation via increased CK2α’ and Fbxw7 activity. Interestingly, muscle tissue analyses of HD mice also expressed increased CK2α’ levels (23). Furthermore, late-stage R6/2 HD mice models were seen to undergo cachexia similarly to late-stage HD patients (24). This finding suggests a link between the PNS and CNS that might explain how progression of mHtt IBs and increased CK2α’ levels within muscle tissues might represent a late dysfunction in HD.

Data show that HSP interaction with mHtt occurs as an attempt to prevent aggregation; however, HSPs that undergo huntingtin sequestration result in widespread chaperone-loss, leading to a substantial increase in misfolded proteins.

**FXTAS, heat-shock proteins and RNA-binding proteins**

FXTAS neuropathology might be mediated by impaired laminA/C and HSPs. LaminsA/C are filament proteins that maintain cell integrity by lining the nuclear membrane. Previous studies have identified the co-localization of αB-crystallin (HSP) and laminA/C within cortex
samples of FXTAS patients (25). Transfection of a FMR(CGG\textsubscript{88})-repeat into neuronal progenitor cells resulted in a 6% increase in IB, where 3% and 5% of them positively stained for lamin A/C and αB-crystallin, respectively (26), suggestive of polyglycine-induced αB-crystallin sequestration. Interestingly, as time progressed, the FMR(CGG\textsubscript{88}) neuronal cells experienced laminA/C nuclear degradation as the lamin ring disappeared. A separate study identified increased mRNA levels of αB-crystallin and HSP27/70 within fibroblast cultures and cortex samples from FXTAS patients (27). A negative correlation between mRNA and protein levels was shown. Surprisingly, analyses of fibroblast cultures from five premutation carriers who had not experienced FXTAS core symptoms displayed no significant differences in mRNA levels (αB-crystallin, HSP27 and HSP70) compared with FXTAS patients (27). Furthermore, premutation mouse embryonic fibroblast (MEF) cells also displayed FMR1mRNA-induced laminA/C abnormalities (27). The association between αB-crystallin, HSP27/70 and laminA/C might reflect an early stress-response to polyCGG. This finding is supported by the fact that αB-crystallin was found to elicit a protective function on sarcomeres upon heat-shock (28). The decrease in HSPs and FMRP mRNAs might reflect sequestration into FMR1 mRNA IBs, inhibiting protein translation. Interestingly, FMR1mRNA expression levels were similar across premutation carriers and FXTAS patients. It was surprising how quickly MEF HD models experienced impaired laminA/C, which suggests that CGG repeats trigger laminA/C disfunction early in FXTAS. The fact that no ubiquitin was detected in the IBs (27), suggests that aggregate formation is independent of the ubiquitin-protease degradation system and that ubiquitin-tagged FMR1mRNAs might be a late event in FXTAS. These findings provide useful data for early diagnosis of premutation FMR1 carriers and a basis for the early neurodevelopmental abnormalities seen in children.

**FXTAS, heat-shock proteins and RNA-binding proteins**

FMR1mRNA-mediated sequestration of Purα contributes to FXTAS pathogenesis. Purα is an RNA-binding protein that regulates RNA-functions (5). PurαKO mice model exhibited a decrease in neuron formation and myelin reduction (29). Although PurαKO did not induce embryonic lethality, 2-week old mice developed severe tremors, suggesting that PurαKO can lead to ataxia (29). Cerebral cortex analyses of PurαKO mice also revealed memory deficits and
decreased neuron levels (41%) (30). Furthermore, due to the young age the mice experienced tremors and Purkinje cell density loss suggests that Purα is required for neuronal development. Apart from Purα-mediated FXTAS pathogenesis, a proteomic approach of transgenic FXTAS fly model(rCGG90) identified Purα co-localization with Rm62, a Purα-interacting protein that mediates rCGG-toxicity (31). Rm62 is involved in HSP70 mRNA exportation. Furthermore, the transgenic flies(rCGG60) displayed a significant decrease in Rm62 protein levels vs. WT flies. Interestingly, a comparison of total RNA from nuclear and cytoplasmic fractions of transgenic and WT flies revealed transgenic nuclear fractions express a two-fold HSP70 transcript increase along with differentially expressed stress genes such as HSPs and attacin (31). These findings suggest Purα-mediated Rm62 sequestration into FMR1mRNA aggregates impairs Rm62-mediated mRNA transport. The increase in stress-response transcripts reflects an initial response to the CGG-stress; however, impairment of Rm62-mediated nuclear exportation interferes with the neuron’s ability to produce proteins that respond to cellular stress. These studies display how Purα, and Purα-interacting proteins become impaired in FXTAS.

FMRpolyG/A aggregates have been identified in FXTAS brain samples. Interestingly, these aggregates stained positively for ubiquitin and HSPs (5,14). Recent studies using nuclear magnetic resonance spectroscopy revealed that Purα binds DNA and RNA the same way (32). Therefore, FMRpolyG/A-induced aggregates formation sequestration of Purα may also contribute to FXTAS.

**Huntington's disease, disruption of axonal transport and neuropathy**

HAP1 (huntingtin-associated protein 1) is a protein involved in intracellular trafficking (33); however, HAP1 becomes impaired in HD, resulting in aberrant axonal transport. Previous studies identified the co-localization of HAP1 within mHtt aggregates, while others have shown mHtt-HAP1 co-localization within microtubules and lysosomal organelles (33). KIF5 (kinesin-related motor protein) is a microtubule-associated protein involved in organelle transport. KIF5 and HAP1 mediate GABA<sub>A</sub>-receptor (GABA<sub>A</sub>R) trafficking. GABA<sub>A</sub>Rs regulate the strength of inhibitory synaptic plasticity (34). Live cell microscopy analysis of transfected mHtt(Q109) neurons revealed a significant decrease in GABA<sub>A</sub>R velocity, clustering and decreased inhibitory post-synaptic currents (IPSC); these are similar findings to purposely inhibited HAP1-KIF5
complex formation, suggestive of mHtt-mediated impaired GABA_A localization. A separate study also identified decreased GABA_A R IPSC in a transgenic mouse model (mHtt-Q_{82}). Interestingly, aberrant IPSCs were observed early during in symptomatic HD model (3–5 months old) and not in presymptomatic mice (35). The symptomatic HD mice developed loss of coordination and gait, which suggests that impaired KIF5-dependent GABA_A R trafficking might contribute to the altered information processing seen in middle-late stage HD. However, another study displayed that presymptomatic 2-month-old mice (Q_{82}) did not experience altered IPSCs (35). It is interesting to consider whether aberrant GABA_A R trafficking might play a role in the epileptic seizures experienced by children with early HD. Furthermore, we cannot exclude the possibility of additional KIFs-HAP1 interactions and their influence on GABA_A R trafficking. Disrupted GABA_A R trafficking to synapses is likely to result in an increased excitotoxic synaptic response, contributing HD pathogenesis. This hypothesis is likely, given a polyQHtt-mediated NMDAr excitotoxicity has been proposed in HD.

**Huntington's disease, disruption of axonal transport and neuropathy**

Normal huntingtin induces the transcription of the brain-derived neurotrophic factor (BDNF), a neuroprotective factor required for MSNs development (36). TrkB is the receptor for BDNF. mHtt and impaired BDNF might explain the neuronal loss and motor alterations seen in HD. Striatal sample analyses from young R6/2 HD mice models displayed normal full-length-TrkB and BDNF expression but significant neuronal degeneration; however, active-phosphorylated-TrkB levels were decreased vs WT mice (36). Furthermore, R6/2 striatal culture exposed to high BDNF-doses displayed minor TrkB and Erk1/2 signaling increase (36); this finding reveals that mHtt impairs BDNF-induced TrkB and Erk1/2 pathway activation. These findings suggest that active TrkB levels are decreased in young HD mice prior any obvious neuronal morphology changes. Downstream defects in TrkB signaling pathways might underlie early MSN deficits seen in HD and result in BDNF reductions and neuronal loss as seen in late-stage HD. Although conflicting reports describe normal BDNF levels in HD brain samples (37), reduced neurotrophic support might not rely only on BDNF. Interestingly, the ERK1/2 pathway promotes cell death by suppressing survival pathways; therefore, attenuated ERK1/2 activity might be representative of an abnormal autophagy in HD with possible links to neuronal death.
Although the exact mechanism by which mHtt interacts with TrkB is unknown, it is known that TrkB is mediated by CREB. CREB binds to a CREB-TrkB promoter site to induce its transcription (39). Downregulation of CREB has been reported in HD (39). Therefore, mHtt impairs CREB-activity, which contributes to aberrant TrkB-BDNF signaling, resulting in neurotrophic support loss and neuronal death. Other proteins shown to interact with mHtt are shown in Table 1.

| Interactor   | Function                        | Consequence of mHtt interaction                                                                 | Reference |
|--------------|---------------------------------|--------------------------------------------------------------------------------------------------|-----------|
| β-Tubulin    | Vesicle transport               | Disrupts intracellular transport and insulin secretion                                             | 53        |
| CBP          | Transcription factor            | Cognitive deficits                                                                             | 54        |
| HIP1         | Regulates apoptosis and gene expression | Transcriptional deregulation                                                                 | 55        |
| NFκB         | Transcription factor            | Results in increase in inflammatory players implicated in disease progression and neurodegeneration | 56        |
| Calmodulin   | Ca\(^{2+}\) regulator          | Increase intracellular [Ca\(^{2+}\)] Results in (+) calpain and causes cytoskeletal and perimembrane protein degeneration | 57        |

Table 1. Proteins interacting with huntingtin protein resulting in impaired protein function.

**FXTAS, disruption of axonal transport and neuropathy**

RNA-binding protein hnRNPA2/B1 is involved in RNA processing and transport (2). Sequestration of hnRNPA2/B1 into IBs might mediate FXTAS pathogenesis. Indeed, insertion of FXTAS patient-derived rCGG\(_{90}\) DNA fragments into *Drosophila* model disrupted eye morphology and induced cell death (40). However, in a separate study, hnRNPA2/B1 overexpression decreased rCGG-mediated eye toxicity, suggesting that hnRNPA2/B1 mediates rCGG-neurodegeneration (41). EMSA analysis revealed that BC1 RNA, an effector of translational control, interacts with hnRNPA2/B1 via a 5′BC1 G-A motif (42). However, introduction of RNA-(CGG\(_{105}\)) significantly reduced dendritic delivery of BC1, suggestive of competitive rCGG-hnRNPA2/B1 binding. rCGG expansions induce conformational changes that form hairpin structures sharing GA motif features (42), resulting in the sequestration of hnRNPA2/B1 into *FMR1*mRNA aggregates. At normal *FMR1*mRNA repeats, hnRNPA1/B2 interaction is regular. Upon CGG-expansion, hnRNPA2/B1 binding increases in favor of aberrant *FMR1*mRNA levels,
thus impairing BC-dendritic delivery. Furthermore, other hnRNPA2/B1-interacting proteins were found to co-localize in rCGG aggregates, including CUBP1. CUBP1 is an RNA-binding protein involved in mRNA stability (42). These finding suggests that rCGG aggregates can directly and indirectly sequester hnRNPA2/B1 and hnRNPA2/B1-binding proteins, thus impairing the transport of regulatory mRNAs and RNAs and seeding aggregate formation. Interestingly, Muslimov et al., (42) questioned whether CGGs mediate RNA targeting. Indeed, transfection of “healthy” CGG24 range into α-tubulin RNA displayed increased dendritic delivery compared with CCC24 transfected repeat (42). This finding indicates that CGGs contain spatial codes for dendritic RNA targeting, which is an important finding given polyglycine expansions with associated spatial codes might explain the increased FMR1 mRNA IBs seen in impaired dendritic signaling. It would be interesting to consider how transient alterations of the localization signals of toxic FMR1 mRNA might reduce dendrite localization and associated neuronal loss.

**FXTAS, disruption of axonal transport and neuropathy**

Sam68 is an RNA-binding protein involved in cellular processes required for hippocampal physiology. A previous study identified Sam68 sequestration into mRNA aggregates in transgenic mice (CGG98) (43). Interestingly, these mice experienced motor defects, suggestive of Sam68 involvement in the tremor and ataxia symptoms of FXTAS (43). Neurexin-1 mRNA is another Sam68-substrate (44). Neurexins are synaptic receptors involved in presynaptic terminal assembly (44). Therefore, impaired SAM68 function might also account for neurexin-1-mediated hippocampal synapse loss. Sam68 domain mapping identified an RNA-binding region that mediates sequestration of other RNA-binding proteins. For example, COS-7(rCGG90) cells induced sequestration of MBLN1 and hnRNP-G, while deletion of the domain abolished aggregate co-localization (43). These findings indicate that Sam68 mediates the sequestration of multiple proteins, sequestering them from their normal functions promoting hippocampal degradation. Upon 24h-post rCGG transfection, Sam68 aggregate co-localization occurred quickly (43), which suggests that Sam68 is an early mediator of aggregate formation which induces sequestration of important proteins. For example, early β-actin sequestration
might explain focal muscle weakness while attention deficits could result from impaired neurexin. Other proteins shown to interact with FMR1mRNA are shown in Table 2.

| Interactor          | Function                        | Consequence of FMR1 mRNA interaction                                                                 | Reference |
|---------------------|---------------------------------|-------------------------------------------------------------------------------------------------------|-----------|
| Drosha/DGCR8        | microRNA biogenesis             | Decreased gene regulation                                                                           | 2         |
| Caprin-1            | RNA-binding protein             | Decreased RNA neuronal translation                                                                  | 58        |
| TRA2A/B             | mRNA splicing regulator         | Splicing defects associated with intellectual disabilities                                          | 55        |
| Transferrin         | Iron regulator                  | Decreased O₂ transport, impaired mitochondrial respiration, impaired CNS signal transduction         | 60        |
| KHDRBS1             | Splicing regulator and mRNA transport | Dysregulation of immune system activity which may result in sustained inflammation and tissue damage and cell death | 61        |

Table 2. Proteins or RNAs interacting with FMR1 mRNA transcript.

**Therapeutic tools and future directions**

**Symptomatic treatment**

Currently there are no treatments for HD and FXTAS, but there are medications that can help alleviate symptoms. TBZ is one of the FDA-approved HD drugs for chorea (45) (Table 3). However, patients taking TBZ have experienced adverse reactions including sleep disturbances and hyperkinesia (45). Previous reports describe positive responses for β-blockers and primidone treatment for FXTAS tremor and ataxia (46). However, reports have revealed them to be effective in six out of eleven patients (46). Although these are somewhat effective symptomatic treatments, further research is required to find a cure for these conditions.

| Drug               | Mechanism of action                      | Outcome                                                                 | Reference |
|--------------------|------------------------------------------|-------------------------------------------------------------------------|-----------|
| Creatine – HD      | Facilitates ATP recycling                 | Improve mitochondria function and decrease muscle wasting Effect varies per patient | 62        |
| Deutetrabzine -HD  | Believed to reverse monoamines from nerve terminals | Decrease chorea symptoms                                                | 63        |
| Laquinimod – HD    | Believed to compromise immunomodulatory effects of T-cells | Increase BDNF, decrease apoptosis and decrease associated-tissue inflammation | 64        |
| Topiramate - FXTAS | Blocks voltage-dependent Na⁺ and         | Reduce tremor Effect varies per patient                                  | 65        |
Gene targeting therapy - Reducing protein and mRNA expression

RNAi utilize synthetic modifiers (siRNA) that bind to target regions for degradation. The potential of adeno-associated virus (AAV)-mediated expression of siRNA has been examined in nonhuman-primate putamen samples (47). The results revealed a significant decrease in striatal mHtt mRNA levels and improved motor functions (47). Although this is a promising result in terms of treatment, a major challenge in the clinical implementation of RNAis is their delivery past the blood-brain barrier. This challenge can be overcome via convection enhanced delivery (CED). In CED, RNAis are synthesized under positive pressure to increase their distribution volume rather than relying on passive diffusion (47). Currently, clinical trials are underway for RNAis such as AMT-130. AMT-130-treated transgenic minipig HD models displayed a significant reduction in mHtt aggregates by 68% and 47% in the striatum and cortex, respectively, 6-months post-treatment (48). Gene targeting therapy for FXTAS is still under study via reductions in toxic FMR1 mRNA and FMRP. However, this method might not be as feasible, given certain FMRP levels are required for normal physiological processes. Therefore, gene targeting therapy that reduces FMR1mRNA build-up represents a conflict between decreasing toxic mRNA but maintaining functional FMRP. Other clinical trials are shown in Table 5.
Many trials are underway in hope to find a cure for TRDs; however, these trials have yet to release their results to the public; thus, drug effect on conditions are unknown.

**Future directions**

Technological advances have enabled the identification of protein-protein and protein-RNA interactions. Understanding the interactions between molecules can aid in elucidating molecular functions and discovery of drug targets. Protein-Protein-Interaction-Optimizer (PIPINO) is a new program that detects protein-protein interactions. PIPINO facilitates the selection of binding partners and compares interaction network dynamics (49). Kalkhoff *et al.*, (49) support PIPINO over co-immunoprecipitation, as such method precipitate background proteins due to non-specific interactions yielding false-positive results. PIPINO can identify potential protein-protein interactions that contribute to HD pathogenesis. catRAPID is a new program that can identify protein-RNA interactions by estimating their binding propensities (50). For example, catRAPID analysis of rCGG identified multiple RNA-protein interactions including co-localizing (hnRNP-G) and non-coloalizing (FMRP) proteins (50). catRAPID results are in agreement with previous data showing co-localization of hnRNPA2/B1 within *FMR1*mRNA aggregates (42). The agreement between in silico and primary data can identify discrepancies between studies, facilitating the identification of mRNA-protein interactions that contribute to FXTAS.

**Future directions**

A new, innovative approach includes CRIPSR/CAS9 genome editing. In brief, an in vitro assay using ipluripotent stem cells from FXTAS patients was combined with CRIPSR/CAS9 in hopes of introducing a DNA double-stranded break and activating repair mechanisms (51). Indeed, a reduction in and removal of CGG repeats were observed and surprisingly, edited neurons displayed reactivation of *FMR1*. CRIPSR/CAS9 is an exciting approach to gene correction for HD and FXTAS. However, further research is required to understand the impact of gene reactivation.

**Conclusion**

HD and FXTAS pathogeneses are mediated by complex interactions throughout the body. Due to the large number of affected intracellular pathways, it is difficult to pinpoint
exactly where the mutation has gone wrong without considering the downstream effects. Our understanding of the pathogenic mechanisms of TRDs has substantially increased in recent years. While many aspects of TRD toxicity remain a mystery, the data analyzed in this review hopes to contribute to our understanding of how protein sequestration impairs the proteostasis network and neurogenesis, thus elucidating how these alterations mediate HD and FXTAS neuropathogenesis. Although no effective therapy for HD and FXTAS has been identified, the aforementioned therapies shed light onto the possibility of finding a cure for these conditions.
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